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Regarding the Courses of Instruction proposed to be given by the Liverpool School of Tropical Medicine, and the Examinations for the Diploma of Tropical Medicine arranged to be held by the University of Liverpool during 1907 (subject to such alteration as may hereinafter be decided upon),

Lent Term begins January 15.
Lent Examination, March 25.
Summer Term begins May 1.
Summer Examination, July 15.
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1906	Chisholm, James Alexander
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1907	Collinson, Walter Julius
1905	Critien, Attilio
1904	Dalziel, John McEwen
1904	Dee, Peter
1907	Donaldson, Anson Scott
1906	Dundas, James
1906	Faichnie, Norman
1907	Fell, Matthew Henry Gregson
1907	Graham, James Drummond
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1907	Hiscock, Robert Carroll
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz
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Date of Diploma	
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1907	Kenrick, William Hamilton
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1906	Mackenzie, Donald Francis
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EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which have been issued since 1899, will be followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the third number.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. Altogether twenty-one Memoirs, besides other works, have been published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905; and there is no reason to suppose that this rate of production by the workers of the School alone will diminish in the future. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

CONCERNING CERTAIN PARASITIC PROTOZOA OBSERVED IN AFRICA

CONCERNING CERTAIN PARASITIC PROTOZOA OBSERVED IN AFRICA*

Being the Eighth Interim Report of the Expedition of the Liverpool School of Tropical Medicine to the Congo, 1903-5

BY THE LATE
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(*Received for publication May 1st, 1907*)

PART II†

MAMMALS (continued)

DOGS

Leucocytozoön.

In films of fresh blood taken from an apparently healthy native puppy in the Gambia free gregarine-like forms were seen which moved slowly in much the same way as do free drepanidia. Intracorpuscular forms were not seen; the stained slides have been mislaid.

From this observation it seems probable that parasites resembling the haemogregarines described in Indian dogs‡ may also occur in the Gambia.

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*We desire to acknowledge our indebtedness to Dr. J. W. B. Hanington for his kind aid in the preparation of the drawings accompanying the present communication, and in the examination of some of the slides of Arthropoda.

†Part I of this paper appeared in Memoir XXI of the Liverpool School of Tropical Medicine.

‡For example, Christophers: *Leucocytozoön canis*, Scientific Memoirs of the Officers of the Medical and Sanitary Department of the Government of India. New series, No. 26, 1905.

BIRDS

Very many birds of widely different species were examined. Only those in whose blood parasitic protozoa were found are mentioned here; several trypanosomes found in Gambian birds are described in a previous publication, Memoir XI of this School.

Proteosoma was never seen. *Halteridium* was very common among the small birds and pigeons examined in the Gambia. In the Congo *Halteridium* was seen only once or twice in small birds and in none of those in which the parasites described below were found.

ASTURINULA MONOGRAMMICA. (A grey hawk common in the Congo Free State.)

One of these birds was shot about midday at Lokandu and its blood was at once examined. In fresh specimens actively moving trypanosomes were seen; dried films were therefore made at once. On studying them parasites of the same type as the *Leucocytozoön* of Danielewsky[1] (Plates XX-XXIII, figs. 48, 72) were seen to be present in large numbers.

A second hawk of this species was shot at Lusambo. Its blood contained a trypanosome of apparently the same species as that observed in the first hawk; the *Leucocytozoön* was not seen (one small slide examined).

Leucocytozoön ziemanni.

Under the name of *Leucocytozoön ziemanni*, Lühe[2] gives a good review of our knowledge concerning this parasite; to his list of references two additional papers[3, 8] should be added, in which the occurrence of similar parasites is reported in America and in Egypt. Although the name *Leucocytozoön* has the priority, its use seems unfortunate, since the parasite to which it here refers is probably parasitic in other cells besides white cells[4, 6] and it has no resemblance to the recently-described, undoubted leucocytozoa of mammals.

Male (figs. 48-61) and female (figs. 62-67) gametocytes of the types fully and finally described by Schaudinn[4] and previously reported by Sacharoff, Ziemann,[5] Laveran[6] and Berestneff[7] were frequent. In addition, many other undescribed forms of the parasite were seen. From the nature and scantiness (four slides) of our material, it is

impossible to attempt a far-reaching study of the parasite. Nevertheless, a fairly complete description is given of some forms constantly observed. This is done with the purpose of insisting on what an excellent object for study this type of parasite is; especially since some of the forms seen seem in some measure to harmonise with Schaudinn's description[4] of the development of *Spirochæta ziemanni* in the blood stream.

Schaudinn gives no particulars but simply states that the development of *Spirochæta ziemanni* differs in no important point from that of *Hæmoproteus noctuæ*.

In our specimens the forms already frequently described as makrogametocytes (figs. 66, 67, 69, 72, 73), and mikrogametocytes (figs. 48, 49) are easily recognisable, and in the main their appearance is in accord with previous descriptions. It is noticeable that in all forms of the parasite the nucleus of the host-cell is but little flattened, and practically never takes the dumb-bell-like shape described by former observers in similar parasites. The position of the host nucleus in the adult parasite varies according to the position in which the parasite dries on the slide. Although it is usually placed laterally at a point about midway between the two extremities of the parasite (fig. 49), it may occur in any position. It has frequently been extruded (fig. 65), and gametocytes without any included host-cell nucleus are not uncommon (fig. 66).

The general structure of the parasites of each sex at all stages of development is the same. Each parasite has periplast, ectoplasm, endoplasm and nuclear bodies. The "periplast" consists of a non-granular, pink-staining (always ?) sheath which invests the whole of the parasite. In it run fairly numerous faint, non-staining, longitudinal, or, if the parasite is twisted, apparently diagonal striations (these can be seen by careful examination in even the youngest parasites). The periplast with its striations is, as a rule, best seen in the more lightly staining mikrogametocytes (fig. 50). In the adult forms the periplast is prolonged at either extremity into long slender processes. When the matured parasite becomes rounded, preparatory to conjugation, the periplastic sheath (fig. 77) is thrown off together with the effete host-cell nucleus (fig. 31).

A similar process probably occurs in the change from the resting to the motile stages (Schaudinn)[4].

Appearances in young parasites (Plate XX), as well as in the

gametocytes, whose effilated extremities have a distinctly granular structure, indicate that an "ectoplasm" exists quite distinct from the periplast. In the young parasite its tendency is to stain blue; in the adult it is pink.

The "endoplasm" is the most striking part of the whole parasite. So much so that it, with the nuclear bodies, has been described by some as the whole of the parasite. In the makrogametocytes the endoplasm stains a deep blue (fig. 73). It frequently contains large numbers (20-30) of coarse, or fine, vividly chromatophilic granules and many small, circular non-staining areas—"vacuoles." The endoplasm of the mikrogametocytes (fig. 49) stains much less deeply, and rarely contains granules or vacuoles. The endoplasm appears to consist of a system of wide alveoli filled with a more or less granular protoplasm. This structure is particularly well seen in the makrogametocytes. The sexual variation in the endoplasm exists, though in a minor degree, in some of the smallest parasites (endoplasm measures 4μ by 3μ).

The form of the "nuclear bodies" varies enormously, according to the sex and stage of development of the parasite.

The amount of chromatophilic material—"nuclear material" in its widest sense—present is much greater in the adult male (fig. 53) than in the female parasites (fig. 67). For convenience of description the various nuclear structures observed at different stages of development are given the following names: "chlamydoplasm,"* "nucleus," "blepharoplast," with an accompanying "vacuole," "the line" and certain "dots," whose nature is certainly problematical. It must be clearly understood that these names are only descriptive and that our material has not enabled us to definitely ascertain the functions of the bodies to which they are applied. The fixation and staining employed are both faulty. The parasites are often distorted by pressure of surrounding red cells and partially destroyed—occasionally useful details can be gleaned from such "fortuitously dissected" specimens (figs. 11, 12, 15, 36). Romanowsky is, unfortunately, a diffuse stain, and the details of dense structures, such as blepharoplasts and the "line," are often hopelessly obscured.

* From Gr. χλάμυς =a cloak. This name is adopted as a descriptive term to indicate the diffusely pink area surrounding the nucleus. It is employed in preference to other terms indicating similar structures since we know nothing, and wish to assert nothing, concerning its function.

The "chlamydoplasm" is a diffuse, light-purple-staining area whose outlines, though very irregular, are, nevertheless, usually definite. In the female parasites (figs. 66, 67, 72, 73) and in males with lines (figs. 33-46) it is comparatively compact. In older mikrogametocytes its mass becomes greatly increased (figs. 50-56) and its outline is less definite (figs. 58, 61). It may lie compactly about the nucleus or stretched out (fig. 76) and distributed in any way through the endoplasm. If it is divided, one part remains with the nucleus, the other is about, or near, the blepharoplast (Fig. 50).

The "nucleus" is employed as an indefinite term to designate the denser collection of chromatin enclosed in the chlamydoplasm. Its shape varies considerably; possibly its nature does so also.

The "blepharoplast" is the term applied to a very deeply staining collection of chromatin usually occurring quite alone in the endoplasm, and showing a distinctly granular structure (figs. 3, 55, 48). In the younger parasites (figs. 3, 5) only two to four granules, arranged in pairs, can be counted. In the older parasites (figs. 68, 71) the blepharoplast consists of one central granule and from six to eight other granules joined together by fine lines and situated about the periphery of the sphere of translucent chromatophilic material in which they are placed, and outside the chlamydoplasm. As a rule, no blepharoplast-like body exists separate from the nucleus in parasites which show the line formation; rarely one is present (fig. 37). In such parasites the line seems to have split at the end opposite to that at which the free blepharoplast is placed. This "blepharoplast" is probably formed by division of the "nuclear" mass of chromatin. An oval non-staining or more lightly staining area, the "vacuole," is very constantly associated with the blepharoplast in parasites of all ages. It may vary greatly in size (figs. 53, 73). "Dots," or chromatic granules similar to those illustrated in figs. 15, 19, 23, 47, 59, were seen in a few instances. They occur in parasites of all sexes and sizes in any position. It is impossible to say anything concerning their function from our specimens. In one parasite of the type illustrated in fig. 71 a chain of five pairs of granules, arranged in a column, occurred within the chlamydoplasm and in immediate connection with the nucleus. In the same way a column of three pairs of granules has been seen placed beside the blepharoplast. In one or two cases a pair of granules was joined by a fine thread.

Unfortunately, our material does not permit a full description and explanation of the developmental phases of these nuclear structures. We can only describe a few of the appearances observed. One process, however, the formation of a "line," seems common to all sexes and ages of the parasite. The line is widest about the middle, and tapers to either extremity. It is usually gently curved; rarely it seems to be slightly wavy. This effect is probably produced by the over-lying striations of the ectoplasm.

We have not determined exactly how this line is produced, nor what is its significance. The morphological changes attending its appearance in the younger parasites are as follows:—The nuclear material, in the youngest parasites (fig. 2, 6), consists of a deeply chromatophilic area, connected with which is a larger pinkish area often containing a few chromatophilic granules. A line is projected apparently from the denser nuclear mass (fig. 1). At the distal end of this line a smaller, densely-staining area appears (fig. 3). The steps intervening between this stage and that shown in figs. 16, 17, 18, where a commencing line is apparently arising from the lighter, not the darker, part of the chromatic material, have not been determined. It is possible that the forms shown in figs. 4, 5, 9, 10 may intervene at this period. In slightly injured parasites, of all ages (figs. 15, 36), it is seen that the line is composed of several (up to four counted) filaments. Sometimes (figs. 28, 33, 33a) it may split longitudinally, apparently normally; and (?) in such cases the nucleus sometimes also divides (fig. 39). Multiplication of the parasite has not been seen. This appearance is the only one observed which in any way suggests division. The line may also divide transversely in the following manner. A portion of the line situated in the chlamydoplasmic area becomes thinned (figs. 26, 32, 40, 41).* An oval pink area differentiated from the rest of the nucleus surrounds this constriction. At either side of the constriction a dark granule develops in the line (fig. 32). Connecting these granules is a very fine dark line or "axial filament." (The "nucleus" in one instance (fig. 26) was connected to one of these granules by a line.)

In another, slightly squashed specimen a filament connected the line with a differentiated "nuclear" area situated in the chlamydo-

* Occasionally the blepharoplast or nucleus may lie over the line and so, through a defect in staining, produce an appearance resembling a constriction.

plasm and then with a "blepharoplast." In some cases the granules and connecting filament can be discerned in the line even before the constriction becomes visible; in two or three specimens a wavy blue line was associated with the "line" (figs. 38, 46, 62). We make no suggestion concerning its nature. The further development of this process was seen in only one specimen (fig. 44), here the two halves of the line are widely separated. The proximal extremity of each is capped by a dark granule and surrounded by diffuse chromatin material, while between them runs the faint axial filament, whose apparent origin has been described.

The process as thus observed seems comparable to the first stages of the formation of motile apparatus in the trypanosome-like stage of *Halteridium (Hæmoproteus) noctuæ*, Schaudinn. The possibility suggests itself that the formation and transverse division of the "line" may represent the third division of nuclear material described by Schaudinn, and that the axial filament may represent the flagellar apparatus of a future trypanosome. Unfortunately, our specimens permit us to go no further than merely to suggest this hypothesis.

Since line formation occurs in parasites of all sizes, it is a process common to all ages of the parasite. This is also true of the development of the trypanosome-like, motile stages of *Hæmoproteus*, and is another point of resemblance between these two processes.

Objections to this interpretation of the line formation are:— (1) None of the smallest trypanosome-like or spirochæte-like stages representing the motile stage of the youngest intracorpuscular parasites were seen. This is strange in a bird so heavily infected. The trypanosome which was present (described below (figs. 29 and 30)), if it has any connection with the leucocytozoön, probably represents the motile stage of a mikrogametocyte.

(2) Forms (figs. 45, 46, 63, 64) in which the length of the line has become much extended do not seem to harmonize with this hypothesis. Advanced stages of transverse division of the line were seen but rarely (figs. 26 and 44 are unique).

The line may lie in any position. It may be connected with its nucleus at its centre or by one end (figs. 45, 74). Although it is usually almost totally in the endoplasm of the parasite, its extremities may extend into the ectoplasm (figs. 39, 46, 74). According to the way in which the parasite has dried on the slide the line may be at

the side of, above, or below the host-cell nucleus. The line is the most resistent part of the parasite, and is often intact when the rest of the parasite has been destroyed in the making of the film.

Young forms, smaller than those illustrated (figs. 6, 7, 8, 13), occur in which it is impossible to detect any structure beyond the presence of a spot of chromatin in a slightly larger area of blue endoplasm. Every intermediate stage occurs between these tiny organisms and such larger, line-bearing parasites as figs. 24, 25. Intermediate stages, without the line, are not seen between parasites of this size and the adult gametocytes. Intermediate stages with the line are frequent (fig. 26). The younger parasites are spherical or have rounded ends (figs. 4, 13, 25); as they become larger their extremities become pointed and affiliated. Many figures in Plate I indicate that the younger parasites are amoeboid. This, and the fact that one or two very young parasites were seen free in the plasm, suggests that the parasites may be able to wander from host-cell to host-cell without the development of a motor apparatus. Such a process is described in *Hæmoproteus noctuæ*.[4] It is evident from changes in form of the adult parasites (figs. 59, 63), apparently not due to artefacts, that they retain up to a late stage something of the plasticity of the younger parasites. The younger parasites seem to apply themselves to (figs. 1, 4, 25) or to enter (figs. 13, 16) the host-cells. The larger parasites engulf them (fig. 26).

It is noteworthy that the parasite's endoplasm is always in close connection with the cell nucleus. As far as staining reaction and appearance goes, it is evident (figs. 6-8, 13, 21, 25) that the host-cells often are mononuclear white cells; very rarely granular leucocytes are attached.

Probably as the infection becomes older, the host-cell, particularly the nucleus, becomes larger and stains deeply (figs. 24, 26, &c). It is noteworthy that the nuclei of the host-cells harbouring female parasites are more often extruded and when present are more degenerated than is the case in the male parasites (figs. 62, 73). Since Romanowsky is a stain which does not penetrate, host-cell nuclei lying beneath the parasite are frequently almost unstained. In such specimens it is often difficult to make out the relative arrangement of host-cell and parasite.

Adult Female forms.

Adult female parasites, makrogametocytes, are rather less numerous and larger than adult male parasites. Their general characteristics, as outlined above, are: deeply-staining endoplasm, containing more or less numerous chromatophilic granules and small clear spaces—"vacuoles"; and small amount of nuclear material, as compared with the male parasites. The average measurements of the most usual type of makrogametocytes (figs. 65, 67) are:—Total length, 55·6 μ; endoplasm, length 20·3 μ, breadth 9·8 μ. Slender forms (figs. 62, 73) in which these measurements were 63·1 μ, 22·5 μ, and 8·5 μ respectively, as well as stumpier forms (fig. 69) measuring 47 μ, 16 μ, and 14 μ, were constant types. Much effilated forms (fig. 72) are rare. They occur most frequently in the thickest parts of our blood smears.

In the most usually seen form (fig. 67) the nuclear material forms a diffusely-pinkish area (chlamydoplasm ?), usually without a sharply-defined limit, but possessing a definite oval contour. In it, or immediately adjacent to it, is the more or less deeply-staining, often granular, blepharoplast (?). Adjoining the blepharoplast an oval, more lightly stained area—the vacuole—can usually be distinguished (fig. 73). Other less conspicuous, dense, chromatic areas may also occur in the chlamydoplasm (fig. 66). The phenomena attending line formation in the female (figs. 62, 63, 64) seem to be analogous to those described in the male and in the young forms; because of the darkly staining endoplasm it is, however, very difficult to follow them.

No changes were observed in the nuclei of the female cells which seem peculiar to them. For this reason no special description is given of them; the few drawings reproduced indicate the resemblance between the nuclear changes in the two sexes. The coarse alveolar structure of the endoplasm, common to all forms of the parasite, is particularly defined in the makrogametocytes. The curved outlines of the alveoli, merging into one another, often give the appearance of wavy blue lines running through the parasite. The granules vary greatly in number and size. There may be as many as fifty. Some are almost dust-like; usually they are larger, and may measure almost ·5 μ in diameter. They frequently occur in pairs and seem to be placed superficially in the parasite. They often lie in lines along the faint striations of the ectoplasm. Of the origin and nature of

these granules we can say nothing certain. Granules of similar appearance sometimes occur in much smaller numbers in mikrogametocytes.

A few rounded forms (fig. 31) are present in which the host-cell nucleus has been extruded and the ectoplasm thrown off. These are possibly parasites prepared for fertilization.[6] Nothing was ascertained concerning the nuclear changes at this stage.* Such parasites measure about 14μ by 9μ.

Adult whet-stone-shaped parasites occur (fig. 70) which possess general characters intermediate between the male and female adult types described. They are, therefore, not readily referable to either sex; but our material does not permit an assertion as to whether an indifferent form exists or not.

Adult Male forms.

Besides the forms in which the line occurs or is developing (fig. 34), there are other parasites where the nuclear material follows a development of different type (figs. 48, 52); it is impossible to say definitely whether there is any connection between these two processes.

Apparently an early stage of this second process is illustrated in figs. 48, 70, 71, 75. There is more or less diffuse and abundant chlamydoplasm. In the chlamydoplasm occurs a denser, at first circular or spherical, mass of chromatin—the "nucleus." (It may rarely be placed just outside the chlamydoplasm.) Outside of the chlamydoplasm, but occasionally in connection with a detached portion of it, is a second denser mass of chromatin—the "blepharoplast." The blepharoplast is identified by its granular nature and by its darker staining. The nuclear material becomes arranged in a thick semicircular arc (figs. 49, 76.) At its centre usually occurs a dot, often connected with the extremities of the arc by fine lines (figs. 53, 55). The arc of chromatin becomes hemispherical and the dot increases in size (figs. 50, 52) until the place of the nucleus is taken by two irregularly oval chromatin masses of approximately equal size (fig. 51). A stage preliminary to this process is possibly illustrated in fig. 47, where four brownish granules, of quite a different colour from the chromatin granules of the blepharoplast, occur in close connection

*The line in the nucleus of fig. 31 makes it very doubtful to what stage this parasite really belongs.

with the nucleus. This specimen is unique; somewhat similar granules once occurred (fig. 59) in connection with the blepharoplast.

It is possible that the division of the nucleus may be by mitosis. Some half-dozen forms like figs. 54, 56, 59 were seen. Unfortunately, it was never possible to distinguish the individual spindle fibres or to count the individual chromosomes. Some spindles, however, had a distinctly fibrillar appearance, and the chromatin was usually very distinctly granular at this stage; in one specimen (fig. 56) the granules could almost be counted—there seemed to be from seven to nine. The further development of this process was not observed.

The type of parasite in fig. 68 is probably an early stage of this second process. The nuclear concentration in the chlamydoplasm is indistinct and circular. The blepharoplast is very well marked; its thread-connected granules are distinct, and not infrequently a chromatophilic granule occurs well outside the blepharoplast, but still connected to it by a well-defined reddish thread (fig. 68). (In one such parasite the blepharoplast had divided into two equal parts connected by a thread.) This apparent extrusion of granules from the blepharoplast occurs, but less frequently, at other stages (figs. 49, 50).

Figs. 51 and 60 are larger parasites apparently undergoing a similar process. In fig. 57 the blepharoplast seems to be extruding two granules, while the nucleus is commencing to divide. In fig. 60 the nucleus, now almost outside the chlamydoplasm, is almost completely divided, while the granules in the blepharoplast have become much more distinct. Fig. 58 is probably a stage in this process. It is suggested that this second process is possibly concerned with the prostages of mikrogamete formation.

A trypanosome.

Six trypanosomes which cannot be identified easily with any parasite already described were seen in the slides containing the leucocytozoön. All were of approximately the same type. The usual measurements were about I,* 3·7μ; II, 10·5μ; III, 1·8μ; IV, 21·4μ;

*The measurements of trypanosomes in this communication were made according to the formula described by us on page 88 of Memoir XXI of this School.

I—Posterior extremity of the parasite to centre of the blepharoplast.
II—From the centre of the blepharoplast to the posterior border of the nucleus.
III—From the posterior to the anterior border of the nucleus.
IV—Anterior border of nucleus to posterior extremity of the body of the parasite.
V—Length of free flagellum.
VI—Breadth of body at its widest part.
The total length of the parasite is also given as a measurement.

V, 11·8μ; VI, 3·7μ. Total length, 49μ. A perfect flagellum was seen only in one parasite (fig. 29). In fig. 30 the flagellum is much shorter; the greater breadth of this parasite is probably due to its being slightly flattened.

The blepharoplast is a very darkly-staining oblong lying longitudinally in the parasite and placed just posterior to an ill-marked vacuole. It obviously consists of a collection of four or more granules. The flagellum, after forming the thickened edge of a wide (1·5μ), ample, lilac-staining undulating membrane, ends in one instance (fig. 30) in a small carmine-coloured, possibly bi-lobed, expansion, in immediate apposition to the blepharoplast. The granular, palely staining nucleus measures about 2 by 1·3μ, and lies in a sharply-defined palely-staining area (3 by 2·5μ), situated rather posterior to the middle of the body of the parasite. In one instance a dark karyosome-like granule, lying in the clear space, is placed close to the nucleus. The nucleus is obscured by the striations of the body, and it is seen with great difficulty. The finely-alveolar body cytoplasm is striated through almost its whole length. The much-pointed posterior extremity (especially in fig. 29) is very lightly stained. Here, as in the finely-drawn-out, darkly-stained anterior extremity of the body, striation can not be detected. At the level of the nucleus about eight, more or less, light striations (myonemes ?) can be distinguished running longitudinally. Ordinarily (fig. 29) the striae are placed at equal distances. In fig. 30 they are so arranged as to make the cytoplasm appear to be arranged in dark striae disposed in pairs.* By careful examination of fig. 30, it was thought that five pairs could be distinguished.

The irregular clear areas in the cytoplasm, as illustrated (figs. 29, 30), occur in the majority of the parasites. They are not thought to be artefacts. They seem to be non-staining, refractile granules rather than vacuoles.

The periplast does not stain as pinkly as is usual in trypanosomes, but it can, nevertheless, be distinguished as a clear refractile envelope about the body of the parasite.

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* Compare with the myonemes in the motile forms of the mikrogametocytes of *Spirochæta ziemanni*.

"BUSH FOWL"

A few trypanosomes, measuring about 50μ in length, were seen in a red-legged bush fowl at Tshofa. Stained preparations were not made. The parasite was remarkable for the extraordinary length and the flagellum-like fineness of its posterior extremity. About one quarter of the total body length lay posterior to the blepharoplast.

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BYCANISTES BUCCINATOR (the trumpeter hornbill).

*Trypanosomes.**

Two distinct trypanosomes, one small and the other large, were seen in a bird of this species shot near Coquilhatville in the Congo Free State. The smaller trypanosomes (Plate XXIV, figs. 1, 2, 3, 4, 5, 6) were by far the most numerous. Their appearance in stained specimens varies very considerably, so that it is possible to describe three distinct types, although forms intermediate between them can be distinguished. These may be called the "slender" (figs. 1, 2), "broad" (figs. 3, 4), and "stumpy" (figs. 5, 6) forms, in accordance with their general appearance. To some extent the structure of these forms varies with their dimensions. The stouter forms usually stain more lightly, have the looser cytoplasm, have no vacuole at the posterior extremity, and their large nuclei do not extend completely across their bodies.

This association of qualities is not constant, however, and all gradations were seen between forms possessing them and the, as a rule, darkly-staining and more compact slender forms. All these forms are, therefore, considered to be merely variations of one parasite, which may be described as follows:—

The blepharoplast stains very densely and more darkly than either nucleus or flagellum. Its position may vary from the extreme posterior extremity, in particular in the "broad" and "stumpy" forms, to a spot 1μ or more from the end of the parasite (fig. 1). It is oblong in shape and is seen to be granular; in several specimens at least four granules, sometimes arranged in pairs, can be counted (fig. 6). Although the arrangement of these granules occasionally suggests commencing division, none of the ordinary longitudinal division forms were seen. The blepharoplast may be placed longitudinally, obliquely, or transversely in the parasite. Just anterior to it there is often a well-defined vacuole, or, when that is absent, a more lightly stained area.

The nucleus frequently extends completely across the body of the parasite and is almost always surrounded by an area stained more lightly than is the remainder of the body. The relation between the size of the nucleus and the type of parasite varies considerably;

* The description of this parasite is reproduced by permission from the Journal of Medical Research. Vol. XVI, No. 5, March, 1907.

as a rule, in the "broad" and "stumpy" forms the nucleus seems relatively larger, of looser texture, and stains more lightly. Chromatic granules, number undetermined, occur in the nucleus of each type of trypanosome (fig. 1).

In several preparations (figs. 3, 6) two small darkly-stained and closely-apposed chromatophilic granules, surrounded by a pinkish area, occur in close connection with the nucleus, but just outside the nuclear membrane. In one instance a well-marked chain of oblong, twin chromatophilic granules runs forward, in an unstained area, from the anterior edge of the vacuole for about a quarter of the distance between the blepharoplast and the nucleus. This structure recalls a similar appearance observed in *Trypanosoma karyozeukton.**

Whether staining deeply or lightly, the cytoplasm of the small trypanosomes is always granular, but the fineness of the granules varies greatly. Well-marked striations of the protoplasm occur in each form, although they are best seen in the broader forms (figs. 3, 4, 5). The striations are evidently a superficial structure, and are usually wound spirally about the parasite. In some specimens a single striation can be followed for almost two complete turns (figs. 4, 5). From their arrangement it is very difficult to estimate the number of striations, but these seem to be about 7 to 8 (figs. 4, 5).

The whole parasite is enclosed in a pink-staining periplast, which can be plainly seen at the posterior extremity and in some parasites all along the edge of the body, where it is unobscured by the undulating membrane (fig. 3). The ample undulating membrane seems relatively widest in the "slender" forms.

The usual dimensions of each form are given in the following table:—

USUAL MEASUREMENTS OF THE SMALL TRYPANOSOME *

		Stumpy Form	Broad Form	Slender Form
Measurement	I ...	1·0 μ ...	1·6 μ ...	1·6 μ
,,	II ...	7·0 μ ...	8·0 μ ...	10·4 μ
,,	III ...	2·0 μ ...	2·4 μ ...	2·0 μ
,,	IV ...	7·3 μ ...	10·4 μ ...	7·2 μ
,,	V ...	7·8 μ ...	8·0 μ ...	9·6 μ
Breadth	VI ...	7·0 μ ...	4·8 μ ...	2·8 μ
Total length	VII ...	25·1 μ ...	30·4 μ ...	30·8 μ

* See page 297

* Dutton and Todd, 1903. Memoir XI, Liverpool School of Tropical Medicine, page 53.

Only two examples of the large trypanosome (total length about 64μ), were obtained. Unfortunately, both are so obscured by surrounding red cells that it is impossible to reproduce them. Their blepharoplast is placed much nearer to the nucleus than to the posterior extremity (and in one instance in a vacuole). The nucleus almost extends across the body. The undulating membrane is ample and the flagellum seems to be comparatively short. The body of the parasite is striated longitudinally; at the level of the nucleus only seven striations could be counted. In one parasite an appearance resembling a longitudinal striation was present in the undulating membrane. The dimensions of this type are: posterior extremity to centre of blepharoplast, 28·3μ (in one parasite only 17μ); centre of blepharoplast to posterior border of nucleus, 3·3μ; anterior border of nucleus to termination of body, 18·3μ; free flagellum, 8·3μ; width 5·8μ.

At Coquilhatville lack of time prevented a careful examination of the parasite in fresh preparations. Some months later another hornbill was shot, and its blood was found to contain trypanosomes resembling the small type described above. Fresh coverslip preparations of blood were kept at room temperature (28° C.) and watched for some hours by Dr. Inge Heiberg. The changes in form indicated in the following diagram were observed to occur in three hours.

Two hours after the preparation was made normal parasites were still seen. Occasionally pairs occurred joined by their flagella. After three hours both normal and very short "stumpy" parasites were seen, while after four hours, longitudinally dividing, spherical and irregular, certainly degenerating, parasites were present.

Eighteen hours after the preparation was made dividing forms, similar to those seen at the fourth hour were still present, while a stouter trypanosome than those seen at the commencement of the observation now appeared for the first time.

REPTILES

A considerable number of snakes, chameleons, lizards, monitors and tortoises of several varieties, as well as three or four crocodiles, were examined in both the Gambia and in the Congo. Parasites were found only in the instances described below.

SNAKES

Trypanosomes.

In stained smears of blood taken from a puff-adder caught in the Gambia, a single trypanosome-like object was seen.

The measurements are as follows:—

Measurements: *	I	... 6·0 μ
	II	... 6·4 μ
	III	... 2·4 μ
	IV	... 21·6 μ
	V	... 8·4 μ
	VI	... 0·6 μ
Total length of parasite		45·0 μ

The body is long, narrow and tapers at each end to a pointed extremity. The body stains a light blue except for two areas between the blepharoplast and the nucleus, which stain dark blue. The first dark area, extending from the blepharoplast anteriorly, is 14μ long; the second, following a short interval, is 3μ long. The nucleus is composed of red chromatin granules and occupies the entire width of the body. The flagellum is a very slender filament. Only a suggestion of an undulating membrane can be seen.

Drepanidia. †

Drepanidia of an ordinary type were frequently seen in the same snake. The nuclei of the cells containing them were displaced, but

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* See page 297.

† In the present state of our knowledge, it seems futile to attempt the identification of a Hæmogregarine from its "drepanidium-like" form alone. For a review of what is known concerning the haemogregarines of amphibians and reptiles, see Lühe; in Mense's "Handbuch der Tropenkrankheiten," page 206. Barth, Leipzig.

not injured; neither was there any destruction of the cell cytoplasm. Two drepanidia were often seen occupying one blood corpuscle, sometimes on either side of the nucleus, and sometimes at one end of the corpuscle, which was then double the usual length (Plate XXV, fig. 52). Two forms of drepanidia were seen, one with a finely-granular, striated, blue-staining cytoplasm (Plate XXV, figs. 50, 55), and the other with a lighter-staining, more loosely-woven cytoplasm, which contained vacuoles, of irregular size and number, at both ends (Plate XXV, fig. 51). These latter forms were often much shorter and wider than the others. The drepanidia measured from 11 to 17μ in length, and from 4·5 to 8μ in width. Free forms of the usual type were seen; multiplication forms did not occur. Folded, "two-shanked," intra-corpuscular parasites were not seen.

A few of the curious bodies illustrated in Plate XXV, figs. 53, 54, and in Plate XXVIII, figs. 56-58, were seen in the blood of this snake. They were rods from 10 to 19μ long and about 1μ wide. They stained homogeneously red with Romanowsky; they were, however, a deeper red at the edges than in the middle. The ends of the rods were somewhat rounded, but never well-defined. In a free single rod there was a deep red dot near the centre (Plate XXV, fig. 53). Two rods were always found in a cell, and they were usually of about the same length, though occasionally one was longer than the other. The rods were sometimes placed parallel, sometimes crossed upon one another, and sometimes came together at one end. They usually occurred in cells which were lighter-stained and had a rounder, redder nucleus. They were sometimes, not always, found in cells containing drepanidia. In two cases free parasites were seen; in one case two rods were found on a degenerated drepanidium; in the other case, a single rod was found free.

Dr. L. Sambon, by a study of slides of blood from other snakes in which these bodies occur more frequently, has shown that they represent the curled-up remains of the capsules vacated by drepanidia.*

An Unidentified Parasite.

A single rounded parasite of the type seen in frogs (page 335) was found in the snake (Plate XXV, fig. 55). It measured 2·2μ in diameter. It contained short red rods arranged, like the spokes of a wheel, about a small central red mass.

* Private communication.

CROCODILES

Trypanosomes.

In stained smears of blood taken from a crocodile (*Crocodilus cataphractus*?) shot in the Congo, a trypanosome with a length of about 35μ (not including flagellum) was seen. The body was about 2μ wide, the blepharoplast was 3μ from the posterior extremity and the nucleus was near the centre of the body and occupied its entire width. The undulating membrane was well developed.

Drepanidia.

Drepanidia of an ordinary type were seen in the same crocodile (Plate XXV, fig. 49). They usually measured 12·5μ in length and 4·5μ in breadth. The nuclei of the host cells were displaced but not injured. No destruction of the cytoplasm of the blood corpuscles was observed, and no division forms were seen. Folded, "two-shanked," intracorpuscular parasites did not occur.

LITERATURE

MINCHIN, GRAY AND TULLOCH. Proc. Roy. Soc., Series B., Vol. 78, p. 251. Report the presence of a large trypanosome in a crocodile in Uganda. They give no further particulars.

TORTOISES

Trypanosomes.

In stained smears of blood taken from a tortoise caught in the Gambia, trypanosomes of one type were seen (Plate XXV, fig. 45). The measurements are as follows:—

Measurement	I	1·2 to 3·5μ
,,	II	7·5 to 18·5μ
,,	III	2·2 to 3·5μ
,,	IV	12·5 to 23·0μ
,,	V	10·0 to 15·0μ
,,	VI	2·0 to 3·5μ
Total length of parasite		35·0 to 58·0μ
Width of membrane		2·0μ

The body tapers gradually towards both extremities, which are sharp-pointed, the anterior extremity being more slender than the

posterior. The cytoplasm is finely granular, and in it are several round unstained spaces 1 to 1·5μ in diameter. In one specimen there were seven of these spaces anterior to the nucleus, one at the posterior edge of the nucleus and four posterior to that. The body has fine longitudinal striations. The blepharoplast consists of at least four granules embedded in a matrix, and is about 1·1μ long; a clear area is in connection with it. From it the well-developed undulating membrane extends in folds to the anterior extremity. The nucleus is round, sometimes slightly longer than wide, and occupies two-thirds of the width of the body. No division forms were seen.

*Drepanidia.**

Drepanidia of an ordinary type were seen in the same tortoise. The nucleus of the host-cell was displaced, but not injured. Two parasites were sometimes seen in one blood corpuscle. No destruction of the cytoplasm of host-cells was observed and no multiplication forms were seen. Some parasites have a cyst wall about them while they are in the corpuscles. This same encysted condition was observed in extra-corpuscular parasites. Many of the drepanidia have coarse, colourless, refractile granules (Plate XXV, fig. 47), either throughout, or only in one half of, their bodies. Two forms of parasites were observed; in one the nucleus stains a dark purple and has a dense structure, as has its cytoplasm (Plate XXV, fig. 46); in the other, which is much larger, the nucleus stains a bright carmine, and the structure of the nucleus and cytoplasm is looser (Plate XXV, fig. 48). The drepanidia measure from 10·4 to 17μ long and from 4 to 5·5μ wide. Multiplication forms were not seen. Folded, "two-shanked" intracorpuscular parasites did not occur.

AMPHIBIA

The only representatives of this class examined were frogs and toads. The blood of several hundreds of these was searched for parasites in either the Gambia or the Congo.

FROGS AND TOADS

Trypanosomes.

In the Congo *Trypanosoma loricatum* vel *costatum*[2] (Mayer, July 1843) was found in the blood of representatives of the following

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* See footnote to page 303.

species of frogs and toads:—*Rana galamensis* (D. and B.), *Rana oxyrhynchus* (Sund.), *Rana mascarensis* (B.), *Rappia marmorata* (Rapp.), and *Bufo regularia* (Renn.). A considerable portion of them was infected. Blood from these infected amphibia was examined fresh and stained. In the fresh examinations, blood was carefully taken aseptically from the heart or, if it was desired to keep the frog alive, either from a leg vein or a toe. Coverslip preparations were made and examined periodically. Blood was kept in sealed capillary pipettes for varying periods and was then used for making films for staining. Blood was taken from the frogs and, with coverslip preparations already made, was examined at all hours of the day and night. Preparations from the organs and bone marrow and of fluid from the body cavities were examined; trypanosomes were seen only in the blood.*

Trypanosoma loricatum was present in almost every frog infected with trypanosomes. It was frequently associated with trypanosomes of any of the types described below. As a rule, it was noticed that parasites of the *Trypanosoma sanguinis*[4] type (Plate XXVI, fig. 27) were associated with *Trypanosoma inopinatum*-like forms and with the leaf-like forms[3] (Plate XXVI, fig. 28); *T. mega*[4] (Plate XXVII, figs. 35-39) occurred with forms resembling *T. karyozeukton*[4] (Plate XXVIII, figs. 40-42) in everything save the absence of the specific chain of granules. In 99 per cent. of the frogs infected with trypanosomes *Drepanidium* was also seen to be present. As a rule, if there were many trypanosomes there were also many drepanidia. Striking exceptions to this rule were, however, seen.†

In fresh blood *T. loricatum* is practically a frilled operculum of protoplasm, somewhat pointed at the posterior end. It is convex on one surface and concave on the other. From the median line of

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* In a single paper Mayer (2) described two parasites under two specific names, *loricatum* (or *costatum*) and *rotatorium*. More recent work has shown that he was probably dealing with two forms of one trypanosome. One of these names must therefore disappear. We retain the name *loricatum* since it was originally applied to a parasite resembling that type of trypanosome which seems to be the adult form of the haematozoon under discussion. A perusal of the present paper makes it evident that various other forms of *T. loricatum* have received specific names. These must also eventually disappear; but until the life history of this parasite has been completely worked out it is scarcely worth while discussing this point.

† For descriptive purposes names already given to various types of trypanosomes are frequently used in this paper. They are used without question to designate parasites resembling those described under these names by the authors quoted.

the concave surface at a little distance from the posterior extremity arises the flagellum. The margins of the organism are roughly serrated. It moves backwards and forwards slowly, now turning over on itself and now bending antero-posteriorly. As many authors have observed, its rate of progression is very slow. It is, therefore, easily watched under the microscope for considerable periods. A side view is seen in fig. 1*, a dorsal view in fig. 2, and a cross section in fig. 3.

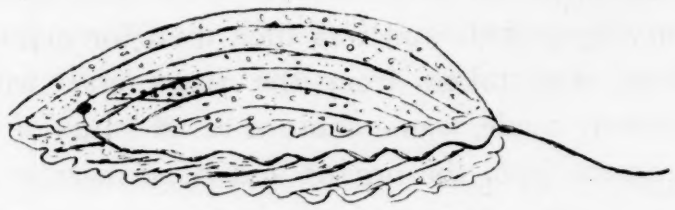


Fig. 1.

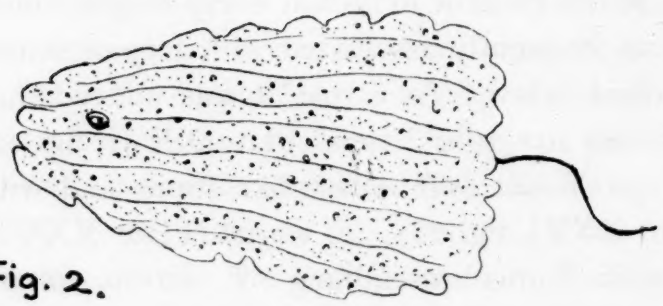


Fig. 2.

Fig. 3.

T. loricatum has a peculiar method of division, which we observed most completely in preparations of fresh blood of *Rana galamensis*, kept aseptically at 72° F. to 89° F. for two or three days.

Amongst recent authors, Franca and Athias[2] and Bouet[6] have seen parts of the cycle described in this paper. Franca and Athias[2] saw representatives of the type of *T. loricatum*, in fresh and stained specimens, become rounded and, in one case, segment several times. In stained specimens they saw a trypanosome of their *T. rotatorium* type become spherical and divide. They describe most interesting nuclear changes in which the blepharoplast seems to play the part of a centrosome, and forms suggesting mitotic division are described. In these same specimens small round parasites, possessing blepharoplast and nucleus, which may develop two flagella, occurred. A small trypanosome of their *T. rotatorium* type was also present.

* The figures in the text are drawn diagrammatically from actual living specimens. Figs. 20 and 24 are enlarged 2000 diameters, the remainder 1000 diameters.

They describe one or two anomalous forms whose position cannot be determined at present. Bouet[6] made cultures from the blood of frogs containing trypanosomes of the *T. loricatum*[2] and the *T. rotatorium*[2] types. The results obtained with either form of parasite were identical. Herpetomonas-like parasites (with an ill-developed undulating membrane, however) were the most usual forms in cultures. It is to be noted that every form of parasite between this type and the trypanosomes originally present in the frog's blood could be seen in the cultures.

Some of the parasites, directly after the preparation was made, were seen to have lost their striations at one or both ends and to have become granular. This process results in a swelling at one or both ends (fig. 4). Later the whole parasite becomes granular; during this

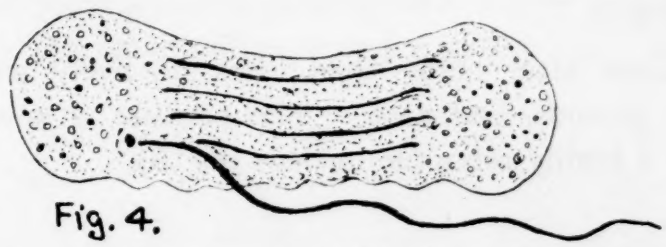


Fig. 4.

process a well-marked nucleus and blepharoplast are present. The body now becomes rounded, and the undulating membrane is gradually peeled off. The flagellum is attached to one end of the organism (fig. 5). The parasite soon becomes completely spherical (fig. 6), and in this condition its diameter is about 24 μ. The flagellum is still

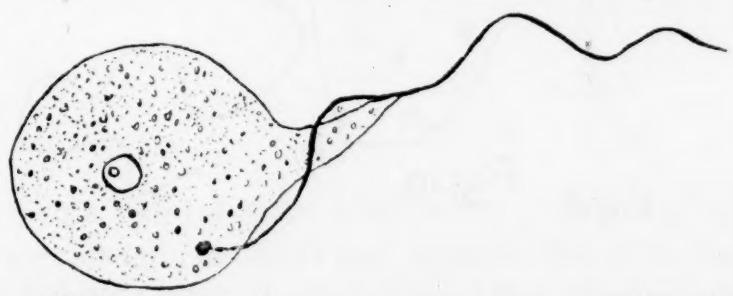


Fig. 5.

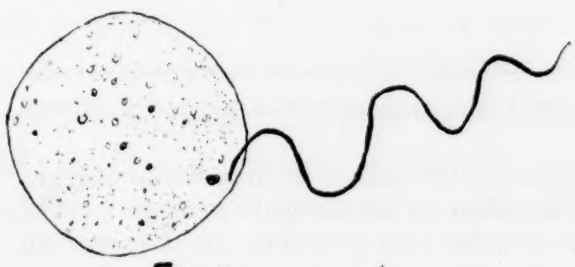


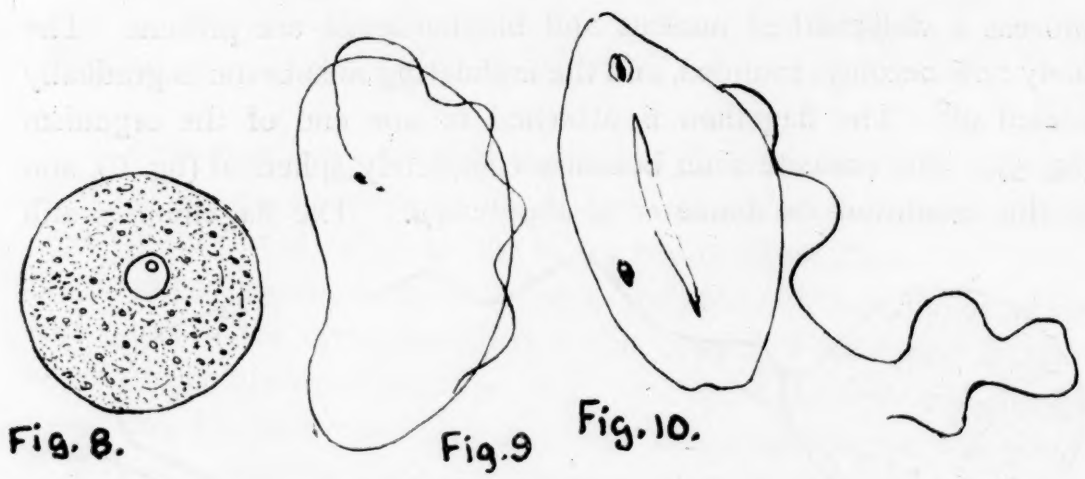
Fig. 6.

Fig. 7

attached, but soon is cast off and is seen lying in the serum, with the blepharoplast attached (fig. 7). Unfortunately, discarded flagella were never seen in stained specimens. It is, therefore, impossible to state whether the whole of the blepharoplast is cast off or not. If the whole of it is discarded a new body strongly resembling a blepharoplast is formed very quickly; because rounded parasites of all sizes from one equalling the original rounded *T. loricatum* to those only 5μ in diameter possess two chromatic areas, one the nucleus proper, the other resembling a blepharoplast.*

It is suggested by some forms that part of the blepharoplast at least may remain when the flagellum is thrown off. Chromatophilic granules not infrequently occur in the cytoplasm of trypanosomes which probably belong to this period. Concerning their nature we can say nothing.

The flagellum after being shed loses its lively movements in three or four seconds, and dies. The parasite is now round and granular with a highly refractile nucleus (fig. 8).



In one observation the parasite lost its striations, became rounded and lost its flagellum in eight minutes after the preparation was made (figs. 9, 10).†

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* It would be expected (2) (8) that the blepharoplast would be newly formed from the nucleus.

† The times given for the periods occupied by the various developmental changes represent actual observations, and may be taken as approximate averages. The same changes may take place more or less rapidly; very frequently the changes take longer than has been indicated.

It then became amoeboid, and in fifteen minutes had moved across two fields (Zeiss, 1·30 aperture, achromatic objective; No. 2 eyepiece). The nucleus became distinctly visible (even before the flagellum was completely cast off), and its structure could easily be made out (fig. 11).

The parasite now elongates (fig. 12), a constriction appears, and almost before division is complete a similar constriction takes place in each of the daughter cells to form four cells, all the cells being almost of the same size as the original mother cell. The four cells divide into eight, the eight into sixteen, the sixteen into thirty-two, and the thirty-two probably into sixty-four.*

Preparatory changes were seen in the nucleus before the first division. These changes were difficult to follow owing to their rapidity. At one point a rapidly-moving little tongue process appears, surrounded by a very small differentiated area (fig. 12). In a second

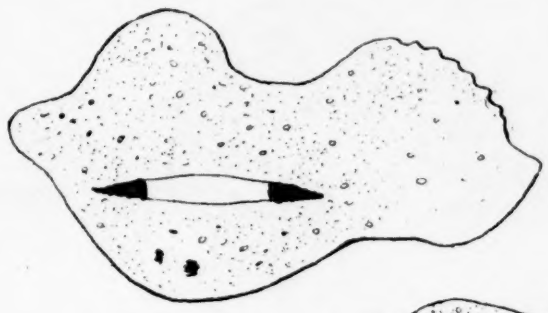

Fig. 11.

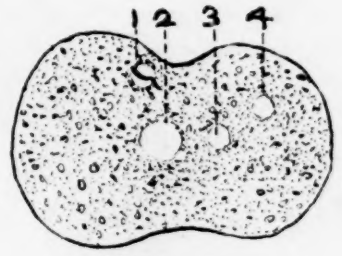

Fig. 12.

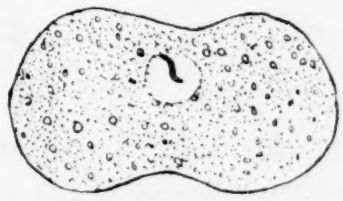

Fig. 13.

or two a dull refractile area becomes visible a little distance from it; a second later another one appears, but it is somewhat smaller, and then still another (fig. 12); but almost before this latter has formed, the four apparently merge into one large, highly-refractile, rounded area in which the little tongue is seen moving (fig. 13). This area is an obvious feature in many of the cells before division is complete, at first very bright in appearance, it gradually fades away; thus in one case it was formed at 3·21, it was much less visible at 3·24, it could not be distinguished at 3·25, and the cells had completely

* Danielewsky states that he has seen 120.

divided at 3·30. Another observation showed the area commencing to be visible at 1·55, very bright after two minutes with the little tongue plainly visible; it was not so bright at 2, and had vanished at 2·03; at this time division of the cytoplasm was well advanced. Another tongue and area was very bright at 2·07, and gone at 2·26. In one case just before its disappearance, the little halo divided, showing a delicate, thread-like connection between two dots (fig. 14). These separated completely into two (fig. 15), and then both disappeared.

A dot was sometimes discernible at one end of the "tongue." Clear areas, smaller and quite distinct from those described above, occasionally appeared for a few seconds in the cytoplasm of the trypanosomes before the first division. One of the products of the first division not infrequently divides before the other. Often one

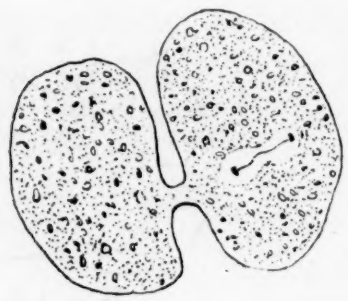

Fig. 14.

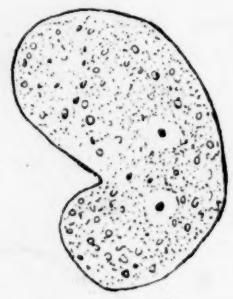

Fig. 15.

or two of the products of the third, fourth or later divisions divides no further. Indeed, the development of the parasite may be arrested at any stage. Such individuals become rounded and very granular and probably usually disintegrate. Some of them may become encysted resistant forms.[6]

In dividing, the cells rotate one upon the other in opposite directions, so that the long axis of one comes to lie at right angles to that of the other (fig. 16). The nuclei were indistinct. The time taken to turn around in one case was about thirty minutes.

In one instance the single rounded organism had divided, in five hours and forty-two minutes, into sixteen cells, which were all apparently inside the outer covering of the original trypanosome (fig. 17). In six hours and a half, many of the cells had divided

again, and in seven hours there were counted thirty-two cells which measured 8μ in diameter. In seven hours and a half, forty-one cells were counted, though there were probably more.

The next change occurring in these cells is the acquirement of a flagellum. Each becomes ovoid, then pear-shaped, and from the more rounded end a flagellum is produced (fig. 18); there are always one or two parasites in each colony which remain spherical and develop no further. The colony of cells now takes on a lively motion. After a while the young trypanosomes become free and

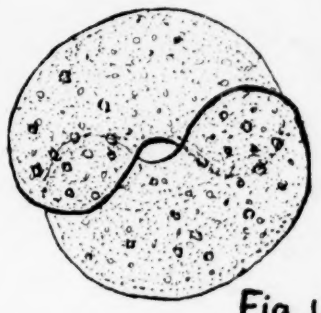

Fig. 16.

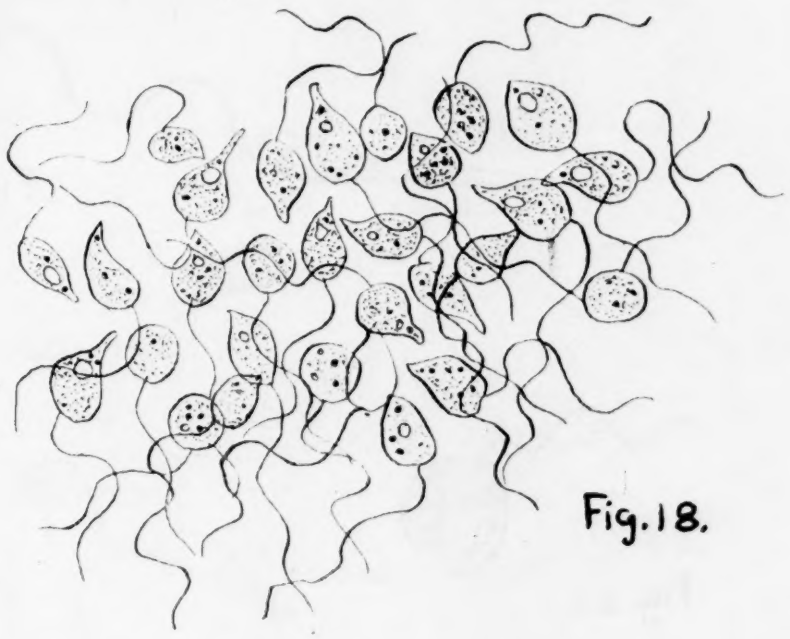

Fig. 18.

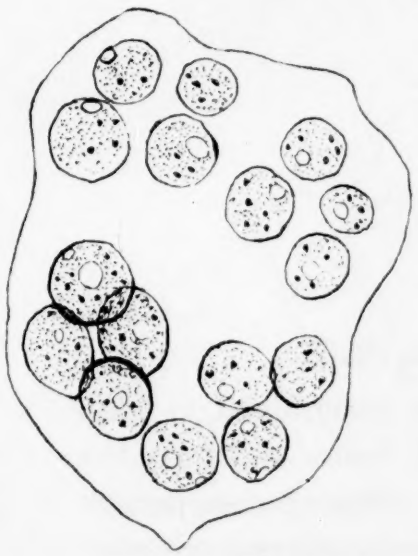

Fig. 17.

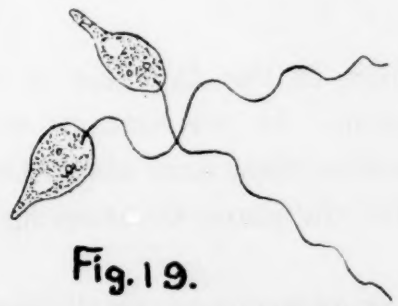

Fig. 19.

their movements increase in rapidity. They divide rapidly by splitting in their longitudinal axis, and thus in the field containing the original cells, and for many fields around are seen large numbers (10 to 15 to a field) of young actively-moving trypanosomes.

The young trypanosomes when first formed from the cell by the acquirement of a flagellum, had the shape and size (× 1,000) indicated

in fig. 19. A large or small vacuole is generally seen near the pointed end. One or two highly refractile dots occur in the cytoplasm. The movements are rapid and take place solely by the lashings of the flagellum, which acts as a tractellum. The cytoplasm does not take part in the production of locomotion even when the parasite has completely separated from its fellows. These small parasites have a herpetomonas-like form; this was especially so after division, when the body of the young parasite is drawn out and its width is almost equal throughout the whole length. No evidence of an undulating membrane was detected in these parasites; nor was the exact

Fig. 20.

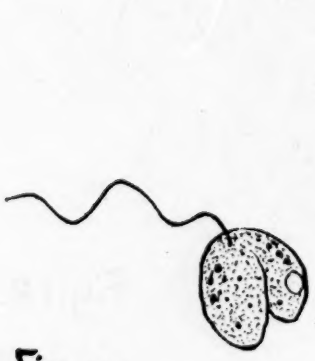

Fig. 21.

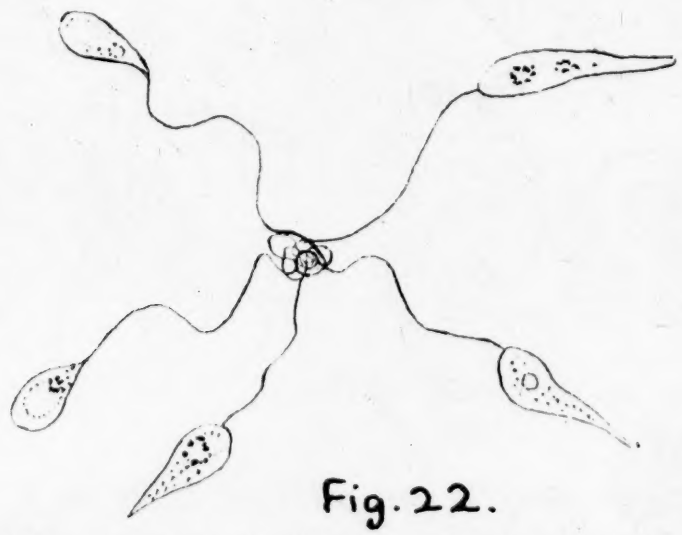

Fig. 22.

termination of the flagellum in the cytoplasm ascertained in fresh preparations. In preparations examined thirty hours and forty-five minutes after they were made, the very active trypanosomes present were all of the shape shown in fig. 20. They contained two refractile areas.

In one preparation small free parasites were seen to divide by becoming rounded. They then divided longitudinally (fig. 21).

In the same preparation examined thirty-two and a half hours after it was made, agglutination* of the small trypanosomes was seen at a spot near the edge of the drop of blood.[6] The parasites were attached together by their flagella (fig. 22). Their movements had slowed down, and later they were absolutely quiescent. Other

* This phenomenon was seen up to 4 days after the preparation of a blood film.

parasites, rounded and attached to one another as in fig. 23, were seen in this situation. These also became motionless.

Four days later in the same preparation these small parasites were still very active; no change having occurred in them, with the exception of the production of a bulb found at the flagellar end of the herpetomonas-like forms[6] (fig. 24). Five days later many small parasites were rounded and granular, but were still moving. Six days

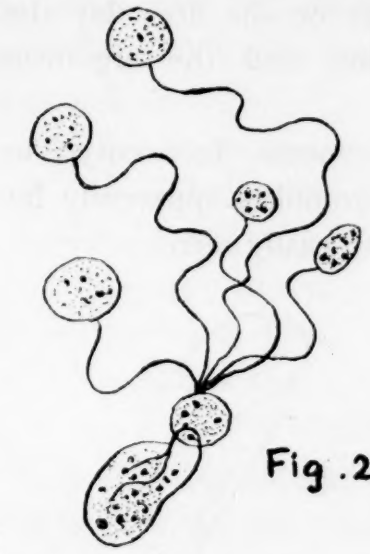

Fig. 23.

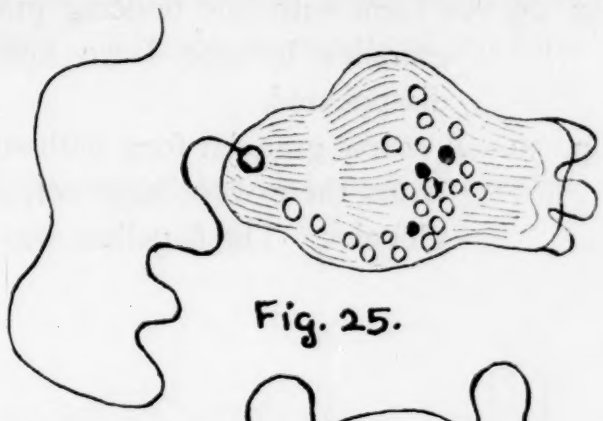

Fig. 25.

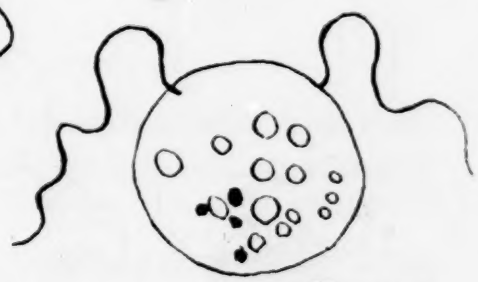

Fig. 26.

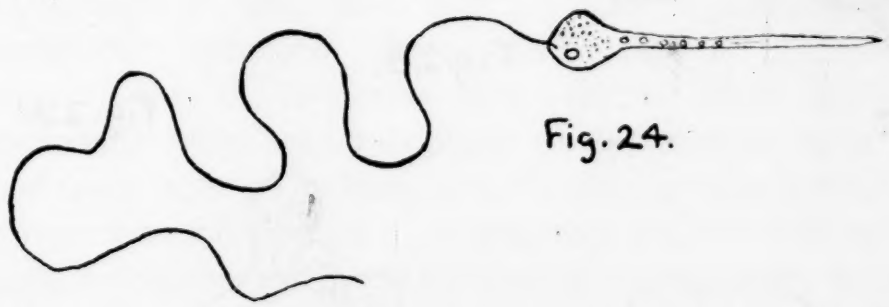

Fig. 24.

later the preparation was discarded, as the small parasites had become rounded, granular and motionless, and the haemoglobin of the red corpuscles was laked.

The next day after the preparation was made a curious looking flagellate was seen. It has large highly-refractile granules, and apparently two flagella; the posterior one moved slowly, the anterior one was fairly active, but the parasite remained stationary (fig. 25). A day later this parasite had become rounded and the flagella were still moving slowly (fig. 26). The following day the parasite had become coarsely granular and the flagella had disappeared.

* Because of their motion and great length it was almost impossible to decide whether there were really two flagella or only one flagellum with a very ample undulating membrane.

Some of the trypanosomes became rounded, but did not develop further than the first or second division. Their outline became indistinct, and, instead of fine granulations, clumps of rather coarse granules appeared, and later showed Brownian movement.

Examples of such parasites watched for five days are as follows:—

Fig. 27.—Shows a coarse granular rounded form.

Fig. 28.—A tri-lobed leaf-like form.

Fig. 29.—A form with fine dancing granules; on the fifth day the granules became large and yellow and the organism disappeared.

Fig. 30.—A coarse granular form without movement. In twenty-four hours there were large refractile granules, apparently fat globules. The flagellum was not so easily seen.

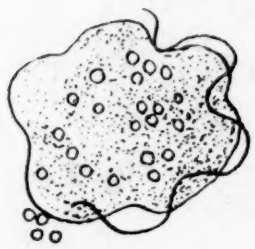

Fig. 27.

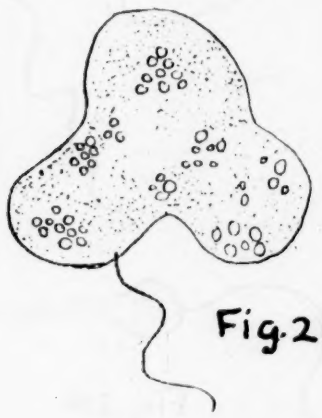

Fig. 28.

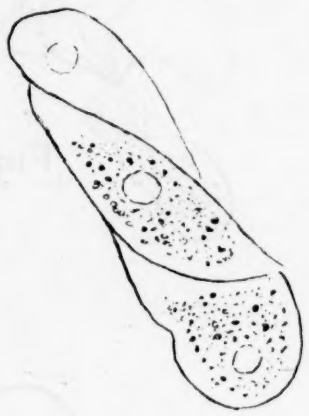

Fig. 29.

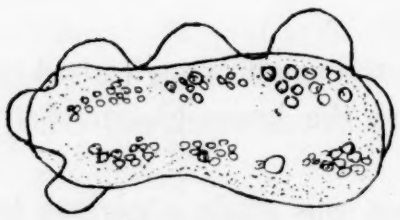

Fig. 30.

Fig. 31.

In one preparation an amoeboid form of trypanosome was seen (fig. 31). It contained a few rather coarse granules. On the second day the granules were somewhat coarser, and the parasite had slightly changed its shape, while the pseudopodia had retracted. On the fifth

day it was obviously degenerated. Some of the herpetomonas-like forms were seen to divide longitudinally. The further development of *T. loricatum* was not observed in fresh preparations.

Stained specimens

From the above-named species of frogs and toads, 220 dried films of blood and organ juices stained by our usual Romanowsky method were examined. *T. loricatum* vel *costatum*[2] was found in most of them (Plate XXV, figs. 1, 2, 3 and 4). The measurements of this form are as follows:—

I.—12 to 42 μ.

II.—The nucleus and blepharoplast are apparently connected.

III.—8·2 to 16 μ.

IV.—23·7 to 26 μ.

V.—8·2 to 16 μ.

VI.—22 to 45 μ.

Total length of the parasite, 52 to 101 μ.

It is a curious coincidence that in the twenty parasites measured, the length of the nucleus is approximately the same as that of the flagellum.

In an organism of this size and shape, spreading and drying in the preparation of the film will inevitably produce a certain amount of distortion (Plate XXV, fig. 1). However, the shape of the *T. loricatum* met with in Plate XXV, fig. 3, corresponds with our description of the parasite seen in fresh blood (page 307). The body is ovoid, the posterior extremity being narrower than the anterior. The organism may be found lying at full length, or with one end partly turned over, or completely doubled upon itself. The structure of the cytoplasm is finely granular. The periplast is pleated into ridges which run longitudinally from the posterior to the anterior extremity (Plate XXV, fig. 4). This appearance can occasionally be seen in the most beautiful manner in slightly disorganised slowly moving parasites, in fresh preparations. Occasionally the organism is twisted or folded upon itself, when the ridges consequently seem to run obliquely. The blepharoplast is usually very small and consists of four or more granules imbedded in a matrix. The thickened edge of the undulating membrane takes its origin from it and the nucleus is connected with it (Plate XXV).

The nucleus in nearly every case is elongated and crescentic, with its concave side toward the thickened edge of the undulating membrane*, and its anterior extremity is pointed. It is 1·6μ to 2·7μ wide. In a very few cases it is round, when its diameter is from 3·3μ to 4·4μ (Plate XXV, fig. 3). The undulating membrane is well developed and runs in folds from the blepharoplast across the middle of the body, from the edge of which it extends about 2·7μ before the free flagellum begins. The structure of the nucleus is more or less complex. Its edges are sharply defined, and at its middle are many very small red granules. The anterior portion of it is dense and finely granular and often contains larger masses of chromatin. In some instances large masses of chromatin are found at both ends (Plate XXV, fig. 2). In the fresh this was found to precede division (see figs. 10 and 11 in text).

Numerous forms occur which have a round body, round nucleus, blepharoplast close to the nucleus and a short white line extending from the blepharoplast, like a short flagellum; this line, however, never extends beyond the edges of the parasite. The line resembles the tongue seen in fresh specimens; both occur in rounded parasites of about the same size, but we are unable to state their identity. It is, however, quite possible that this type of parasite may be a developmental form occurring at about this period and that it may be a product of the first dichotomous division of *T. loricatum*. Differentiated areas of obscure nature are sometimes seen in the nucleus, but in none of our specimens have we observed the interesting nuclear changes described by Franca and Athias.[2]

The observations by Moore and Breinl[8] seem to be of interest in connection with the peculiarly elongated "nucleus" of many forms of *T. loricatum*.

In stained smears of kept blood from the same frog, the same cycle of multiplication and development can be followed as mentioned above in freshly made preparations of fluid blood. In Plate XXV, fig. 8, is shown a parasite rounded and about to cast off its flagellum.

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* The resemblance of this parasite to those described under other names than *T. loricatum* by Martin (8) in an African lizard, and by Marchoux and Salimbeni (5) in a frog (*Hyla*) is very evident. The parasite described by the latter authors is peculiar in that it had no free flagellum (the flagellum of *T. loricatum* is often stained with difficulty, or may lie out of sight beneath the body of the parasite), and its undulating membrane was commenced by a rigid spur which was observed in *T. loricatum* neither by Franca and Athias (2) nor by ourselves.

The body measures 18μ by 13μ, and the free flagellum was 45μ long The blepharoplast is of the usual structure with the usual clear space about it, and from it extends the nucleus as a band 1·1μ wide and 9μ long.

In Plate XXVI, fig. 14, is shown a round form with the flagellum cast of. The cytoplasm is coarsely granular and the blepharoplast which is of the usual structure is situated near the edge of the body. The nucleus adjoins the blepharoplast on one side. The diameter of the organism is 22μ. The nucleus is about 3·3μ in diameter.

A division, probably the first of a round form, is seen in Plate XXVI, fig. 15. The dividing parasite measures 19μ by 13μ. A group of 16 parasites is shown in Plate XXVI, fig. 18; each of these cells measures about 3·3μ in diameter. Other small parasites, probably representing parasites of a third division (Plate XXVI, figs. 16 and 17), have a diameter from 4·4μ to 6·6μ. Plate XXVI, fig. 15, is a division of forms with two blepharoplasts and two nuclei. Such parasites were frequently seen. Occasionally round cells are found with multiple nuclei and blepharoplasts, but with the cytoplasm undivided (Plate XXVI, fig. 19). Such cells were from 11 to 22μ in diameter.

The small round cell now develops a flagellum from the blepharoplast, and the body elongates in one axis (Plate XXVI, fig. 20). This parasite measures 6·6μ by 5·5μ, and has a free flagellum 22·8μ long. The blepharoplast is 0·8 by 0·3μ, and is 1·1μ from the edge. The nucleus is 1·1μ by 1·6μ.

The next stage in the development is the formation of herpetomonas-like forms (Plate XXVI, fig. 21). These have a body length of from 9μ to 22μ, divided as follows:—Posterior granule (see below) to posterior extremity 0·5μ to 4·4μ, posterior granule to nucleus 4·2 to 10μ, length of nucleus 2 to 4·4μ, nucleus to blepharoplast (when blepharoplast is anterior) 1·2μ, length of blepharoplast 0·5 to 0·8μ, blepharoplast to the anterior extremity 2·2μ to 5·5μ. The flagellum is from 10μ to 25μ. The width of the body is 1·2μ.

The body is narrow, tapering at both ends to a fine point.

There is no undulating membrane (Bouet[6] describes one as being present). There are from 4 to 16 vacuoles lying between the posterior granule and the blepharoplast. The blepharoplast of the usual granular type lies either at one side of the nucleus or slightly anterior to it. From it the flagellum arises and runs through the

middle of the anterior portion of the body, or, turning a little to one side, is closely applied to the margin of the anterior part of the body. In one specimen a bluish line connected the blepharoplast with the nucleus. The nucleus is long and narrow, sometimes extending completely across the body, sometimes occupying half the width. Just posterior to the nucleus is a vacuole, and often when the nucleus does not occupy the whole width of the body there is another beside it. Near the posterior extremity is a red mass, rather more lightly stained than the blepharoplast, which consists of from one to four granules imbedded in a matrix; this we have called the posterior granule. The size of this mass varies from a small point 1μ in diameter. In one case a blue line was seen to extend from this mass anteriorly, but only for a short distance. It did not connect the posterior granule and the nucleus. One of these herpetomonas-like forms has a large anterior end (Plate XXVI, fig. 23), similar to the bulbous form seen in the fresh preparations (fig. 24 in text). Masses of agglutinated parasites of this type were seen (fig. 22). Two anomalous forms were seen which probably belong to this state. One has a body length of 10μ and a width of $4{\cdot}4\mu$. Numerous filamentous flagella seem to arise from the blepharoplast at one end, and the organism seems to be encircled by an undulating membrane. The other is quite herpetomonas-like except for the presence of four flagella (Plate XXVI, fig. 22). It measures as follows:—Posterior extremity to posterior granule $1{\cdot}2\mu$; posterior granule to nucleus $5{\cdot}4\mu$; length of nucleus $3{\cdot}3\mu$; nucleus to anterior extremity $1{\cdot}2\mu$. The anterior part of the body in the region of the nucleus and blepharoplast is wider, being $2{\cdot}2\mu$ wide. The flagella are 15μ, $4{\cdot}4\mu$, $4{\cdot}9\mu$ and $5{\cdot}5\mu$ respectively.

Similar forms were seen in the fresh preparations. They bear some resemblance to the trichomonas described in the intestine of frogs. The blood was, however, taken and kept with every care, and there was certainly no contamination. These forms certainly occur in the blood; whether they represent a stage in the development of *T. loricatum* or not it is difficult to say.

The next stage in the development is a form resembling *T. inopinatum*[3] (Plate XXVI, figs. 25, 26). This stage is found in fresh blood, in contradistinction to the forms just described, which were found in kept blood alone. The parasites of the *T. inopinatum* type may be divided into two groups to facilitate measurements. The

first group has the blepharoplast close to the nucleus. Its measurements are:—

Posterior extremity to nucleus	5·5 to 10·0μ
Measurement III	1·6 to 2·7μ
„ IV	11·0 to 20·0μ
„ V	11·0μ
„ VI	3·3 to 4·4μ
Total length of the parasite	28·5 to 43·0μ
Width of nucleus	1·6 to 2·2μ

The other form of *T. inopinatum* has the blepharoplast posterior to the nucleus, and measures as follows:—

Measurement I	·8 to 12·0μ
„ II	2·0 to 4·4μ
„ III	2·0 to 2·2μ
„ IV	13·2 to 16·0μ
„ V	12·0μ
„ VI	1·6 to 3·3μ
Total length of the parasite	32·5 to 49·0μ
Width of nucleus	1·6μ

The body of both forms is pointed at both ends, or is sometimes somewhat blunt at the posterior end. The blepharoplast* is of the usual structure and is situated in a plane common to the nucleus, or posterior to it. The nucleus is round and sometimes occupies the whole width of the body, and sometimes not. The posterior part of the body tapers gradually, and at the level of the blepharoplast the body is widest. The undulating membrane is never very full in these forms.

Forms resembling *T. sanguinis*[4] (Plate XXVI, fig. 27) are present. They seem to be a further development of *T. inopinatum*, since every gradation exists between these two forms. The undulating membrane is the most striking feature. Its thickened edge arises from the blepharoplast and extends transversely across and beyond the body to border the wide membrane. The blepharoplast is in the same plane as the nucleus. The measurements are as follows:—

* That is, it consists of 2 to 4 or more granules embedded in a matrix and surrounded by a clear area.

middle of the anterior portion of the body, or, turning a little to one side, is closely applied to the margin of the anterior part of the body. In one specimen a bluish line connected the blepharoplast with the nucleus. The nucleus is long and narrow, sometimes extending completely across the body, sometimes occupying half the width. Just posterior to the nucleus is a vacuole, and often when the nucleus does not occupy the whole width of the body there is another beside it. Near the posterior extremity is a red mass, rather more lightly stained than the blepharoplast, which consists of from one to four granules imbedded in a matrix; this we have called the posterior granule. The size of this mass varies from a small point 1μ in diameter. In one case a blue line was seen to extend from this mass anteriorly, but only for a short distance. It did not connect the posterior granule and the nucleus. One of these herpetomonas-like forms has a large anterior end (Plate XXVI, fig. 23), similar to the bulbous form seen in the fresh preparations (fig. 24 in text). Masses of agglutinated parasites of this type were seen (fig. 22). Two anomalous forms were seen which probably belong to this state. One has a body length of 10μ and a width of $4{\cdot}4\mu$. Numerous filamentous flagella seem to arise from the blepharoplast at one end, and the organism seems to be encircled by an undulating membrane. The other is quite herpetomonas-like except for the presence of four flagella (Plate XXVI, fig. 22). It measures as follows:—Posterior extremity to posterior granule $1{\cdot}2\mu$; posterior granule to nucleus $5{\cdot}4\mu$; length of nucleus $3{\cdot}3\mu$; nucleus to anterior extremity $1{\cdot}2\mu$. The anterior part of the body in the region of the nucleus and blepharoplast is wider, being $2{\cdot}2\mu$ wide. The flagella are 15μ, $4{\cdot}4\mu$, $4{\cdot}9\mu$ and $5{\cdot}5\mu$ respectively.

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Measurement III	1·6 to 2·7μ
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„ V	11·0μ
„ VI	3·3 to 4·4μ
Total length of the parasite	28·5 to 43·0μ
Width of nucleus	1·6 to 2·2μ

The other form of *T. inopinatum* has the blepharoplast posterior to the nucleus, and measures as follows:—

Measurement I	·8 to 12·0μ
„ II	2·0 to 4·4μ
„ III	2·0 to 2·2μ
„ IV	13·2 to 16·0μ
„ V	12·0μ
„ VI	1·6 to 3·3μ
Total length of the parasite	32·5 to 49·0μ
Width of nucleus	1·6μ

The body of both forms is pointed at both ends, or is sometimes somewhat blunt at the posterior end. The blepharoplast* is of the usual structure and is situated in a plane common to the nucleus, or posterior to it. The nucleus is round and sometimes occupies the whole width of the body, and sometimes not. The posterior part of the body tapers gradually, and at the level of the blepharoplast the body is widest. The undulating membrane is never very full in these forms.

Forms resembling *T. sanguinis*[4] (Plate XXVI, fig. 27) are present. They seem to be a further development of *T. inopinatum*, since every gradation exists between these two forms. The undulating membrane is the most striking feature. Its thickened edge arises from the blepharoplast and extends transversely across and beyond the body to border the wide membrane. The blepharoplast is in the same plane as the nucleus. The measurements are as follows:—

* That is, it consists of 2 to 4 or more granules embedded in a matrix and surrounded by a clear area.

Posterior extremity to nucleus	4·4μ
Measurement III	1·1 to 2·2μ
„ IV	12·0 to 19·0μ
„ V	11·0 to 12·0μ
„ VI	1·1 to 2·7μ
Total length of the parasite	30·2 to 39·0μ
Width of undulating membrane	2·7μ

From *T. sanguinis* there seems to be every gradation to the "leaf-like" trypanosomes of Plate XXVI, fig. 28. These forms occur as frequently as does *T. sanguinis*, and are never seen in slides from which *T. sanguinis* is absent. The posterior part of this leaf-like form tapers toward its round and blunt extremity. This portion stains more deeply and seems to consist of folds tightly folded. At about the junction of the posterior and middle thirds of the body is the round nucleus, and in a common plane is the blepharoplast. The folds of the posterior part of the body gradually unfold until about the middle of the body, where they are completely unfolded. The body then tapers to a sharp pointed anterior extremity. The thickened edge of the undulating membrane arises from the blepharoplast, crosses the body obliquely and continues along the edge of the body at a distance of 1μ from it to the pointed extremity where it becomes the free flagellum. The undulating membrane is not well developed, but the thin edge of the body and anterior portion of the body seemed, in the fresh, to act as an undulating membrane. The measurements are:—

Posterior extremity to nucleus	5·5 to 10·0μ
Measurement III	1·1 to 2·7μ
„ IV	13·2 to 26·0μ
„ V	9·0 to 11·0μ
„ VI, at a level of nucleus	6·6 to 9·0μ
Total length of the parasite	30·0 to 48·0μ

Division forms of this stage were seen.

There was another variety of the leaf-form. The posterior part is serrated at the extremity and the serrations seem to be the points of a petal-like arrangement of the posterior end. The general effect is that of a bud about to open (Plate XXVII, fig. 29).

The leaf-like form seems to gradually change to the second variety of *T. loricatum* described by Franca and Athias.[2] The change is brought about by a shortening of the anterior part of the body and a complete unfolding of its posterior portion. Forms representing this transformation are seen in Plate XXVII, figs. 30 and 31. This completes the cycle from *T. loricatum* to *T. loricatum.**

The first part of this cycle, from *T. loricatum* to the herpetomonas-like forms, was actually observed in a single living parasite which was kept under observation during three days. The second part of the cycle, from the herpetomonas-like forms back to *T. loricatum* is largely based upon the examination of stained preparations of fresh and "kept" blood. We are fully aware of the necessity for caution in determining a developmental process from stained specimens. In the present instance the deductions we have made from our examination of stained preparations have been frequently confirmed, and even supplemented by isolated observations on fresh specimens. Our statement is supported by the observation of Bouet[6] who saw every intermediate stage between rounded forms and adult trypanosomes of the *T. rotatorium* type[2] in his cultures.

We describe several types of trypanosomes which are constantly seen. All are simply developmental variations of *T. loricatum.*

I.—Forms resembling the trypanosome found in *Hyla arborea.*

(*a*) A short variety (probably identical with *T. rotatorium*)

(*b*) A long variety.

(*c*) A wide variety.

II.—A lanceolate form resembling somewhat a type described by Laveran and Mesnil.[3]

III.—Forms resembling *T. mega.*[4]

(*a*) *T. mega.*

(*b*) A coarsely reticulated form.

(*c*) A form with large red granules in it.

IV.—Forms resembling *T. karyozeukton.*

V.—An unplaced trypanosome.

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* Gaule (2) evidently saw a considerable part of this cycle, since he believed that the trypanosomes were produced from the white blood corpuscles. He stated that he had seen leucocytes each develop an undulating membrane and a flagellum. He also describes trypanosomes which cast off their motile apparatus and so again became leucocytes. The way in which such a mistake could arise is very apparent. A clump of rounded parasites at the fourth or fifth division bears a very close resemblance to a group of white cells.

I.—(*a*) A typical short form resembling that found in *Hyla aborea* (Plate XXVI, fig. 10) is about 25μ long, 9μ wide and has a flagellum 28μ long. The blepharoplast is situated 2·3μ from the posterior extremity, and 1·2μ posterior to the nucleus. The nucleus is 9μ long. The chromatin was collected in masses at either end of the nucleus, or was diffusely distributed in fine granules throughout its extent. The cytoplasm is a rather loose network, and it seems to be continued into the undulating membrane. The side of the body carrying the undulating membrane thus appeared to be folded into a series of foot-like projections produced by the involutions of the thick and substantial membrane.

This general description applies to the other two forms.

(*b*) The long form is characterised by its great length, 40 to 63μ, not including flagellum which is from 19·2 to 26μ long (Plate XXV, figs. 6 and 7), its comparatively narrow body, between greatest projections 5·5 to 7·2μ, and the appearance of folds or pleats running longitudinally; this was especially marked at the posterior end. Sometimes the body is particularly thick and dark-stained. The length of the nucleus is 22 to 24μ, and the width is 1μ.

(*c*) The wide form (Plate XXVI, fig. 9) looks like the narrow varieties, with the folds unfolded however. It measures as follows:—

Measurement	I	22·0μ
,,	II	Blepharoplast is connected with the nucleus.
,,	III	22.0μ
,,	IV	19·0μ
,,	V	14·8μ
Total body length		63·0μ

All the "Hyla forms" have the thickened edge of the undulating membrane running about 1μ from the wavy edge of the body. A round nucleus was never found in any of these forms.

II.—The lanceolate variety (Plate XXV, fig. 5) of *T loricatum* has a rather wide and round posterior extremity, from which the body gradually tapers toward the pointed anterior extremity. The body has smooth edges on both sides. The undulating membrane, which is well developed, runs first directly backwards and then curves around the end of the body to run in folds along the median line of the body. There is no cytoplasm in the undulating membrane, as seems to be the case in the preceding "Hyla forms" variety.

The nucleus, a long narrow tube tapering at both ends, especially anteriorly, extends from the blepharoplast toward the anterior end. The length of the nucleus was almost the same as that of the flagellum.*

The measurements of this form are as follows:—

Length of body, 38 to 50μ; width, 10 to 16μ; blepharoplast to posterior extremity 2·2 to 4·4μ; width of nucleus 1·2 to 2μ; length of nucleus 19·8 to 29μ; flagellum, 17·6 to 27μ.

A change in the form of a "leaf-like" trypanosome observed in a fresh preparation is illustrated in figs. 32, 33, 34. The parasite was

Fig. 32.

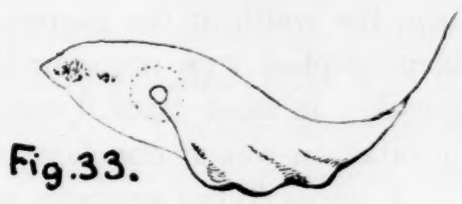

Fig. 33.

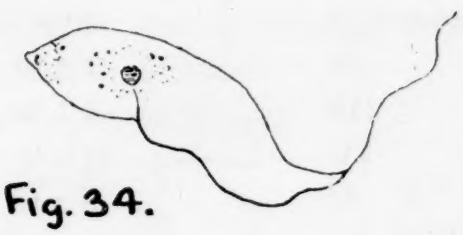

Fig. 34.

watched until it had assumed an appearance, always without well-marked striations, almost identical with *T. mega*. A single form of this type, probably identical with fig. 33, was seen in the stained preparations.

Its measurements are as follows:—

Measurement	I	...	... 9·0μ
,,	II	...	... 2·2μ
,,	III	...	... 4·4μ
,,	IV	...	... 20·0μ
,,	V	...	... 9·0μ
Total length of parasite			... 45·0μ

We look upon this form as an intermediate stage between the "leaf-like" forms (see above) and *T. mega*.[4]

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* It is a curious fact that in *T. loricatum* (type) and in two of the varieties described (Ib and II, see page 323) the length of the nucleus was the same as that of the flagellum.

III.—(*a*) The "*Mega*" forms may have long anterior and posterior (Plate XXVII, fig. 39) ends, a contracted posterior extremity (Plate XXVII, figs. 35 and 36), or a short contracted body (Plate XXVII, fig. 37). In fresh preparations the striations are well seen in the long forms;* these may coil up exactly as does *T. karyozeukton* (Plate XXVIII, fig. 43) (see below). *T. mega* has been seen to gradually become more rounded until it became spherical. It then lost its flagellum. The forms with contracted posterior extremity and contracted body (Plate XXVII, figs. 35-37) probably represent stages in this process. From them development was not observed.

In the type of *T. mega* with a short contracted body the length is 40μ, the width at the posterior part 20μ, the nucleus 3·3μ, and the blepharoplast 1·1μ posterior to it. The width of the nucleus varies greatly; in most cases it extends completely across the body, while in others it was at one side and only 2·2μ wide.

T. mega (type) measures as follows—

Measurement	I	... ...	4·0μ
,,	II	... ...	1·3 to 6·0μ
,,	III	... ...	1·1 to 4·4μ
,,	IV	... ...	15·0 to 53·0μ
,,	V	... ...	8·2 to 16·0μ
,,	VI	... ...	3·3 to 11·0μ
Total length of parasite		...	52·0 to 104·0μ
Width of nucleus		... ...	2·2 to 9·0μ

The original description of *Trypanosoma mega* holds good for these parasites. In a few parasites there was a slight variation in the structure of the nucleus. The clear area just anterior to the nucleus was still present, but the nuclear area of irregular chromatin was replaced by an arrangement of about eight or nine processes which radiated from a focus placed at the middle of the anterior wall of the nucleus. They seemed to be placed in a deep purple stroma, and some of them contained a few reddish granules. A somewhat similar appearance is illustrated by Broden.[7]

(*b*) There was another form similar to the "Mega" type except for the very coarse reticular structure of the cytoplasm (Plate XXVII,

* It may be stated here that more or less distinct longitudinal striations were present in every type of trypanosome mentioned in this paper.

fig. 38). Other forms were quite different. Only five of these well-marked forms were seen. The measurements are as follows:—

Measurement	I	...	8·0 to 11·0μ
,,	II	...	5·6 to 10·0μ
,,	III	...	2·2 to 2·7μ
,,	IV	...	15·4 to 21·0μ
,,	V	...	13·2μ
,,	VI	...	3·0 to 8·0μ
Total length of parasite		...	44·0 to 58·0μ
Width of nucleus		...	2·2μ

(*c*) Another form, seen in both fresh and stained preparations, which resembles *T. mega* is a wide trypanosome with large granules scattered through its body. Some had many granules (Plate XXVII, fig. 34), others had few (Plate XXVII, fig. 33). Usually the granules stained a bright red, some of them, however, were refractile and remained unstained. Although the other granules in the same parasite were stained, we can say nothing concerning the origin of these possibly chromidial granules.

These forms measure as follows:—

Measurement	I	...	9·0 to 15·0μ
,,	II	...	4·4μ
,,	III	...	4·4 to 5·0μ
,,	IV	...	18·0 to 27·0μ
,,	V	...	7·7μ
,,	VI	...	6·6 to 10·0μ
Total length of parasite		...	43·5 to 59·0μ

The nucleus in these forms is always pale, and across it can be seen the striations of the body. The blepharoplast is 1·2μ long, rather narrow, and almost hidden by the large granules, which are about 1·5μ in diameter.

IV.—A trypanosome resembling *T. karyozeukton*[4] is present, but the characteristic chain between blepharoplast and nucleus can never be seen. These trypanosomes may be classified, according to the size, as large, medium, and small, or, better, narrow.

The large form (Plate XXVIII, fig. 42) is sometimes coiled more or less tightly (Plate XXVIII, figs. 43 and 44). This phenomenon was also observed in fresh preparations. The further development of these

forms was not seen. The edge carrying the undulating membrane has a wavy outline. The width of the body at the level of the nucleus is less than just anterior or just posterior to it.

The narrow form (Plate XXVIII, fig. 40) was seen in one case to have a blue line running from blepharoplast spirally to the posterior extremity, and the undulating membrane was continued for a short distance posteriorly as a ridge beyond the blepharoplast. The medium form is seen in Plate XXVIII, fig. 41.

All the forms have clear spaces around the nucleus and blepharoplast.

		Large	Medium	Small
Measurement	I	13·2 to 26·0μ	10·0μ	9·0 to 20·0μ
,,	II	7·1 to 13·0μ	6·6μ	3·8 to 8·5μ
,,	III	3·3 to 5·5μ	3·3μ	2·2 to 3·5μ
,,	IV	51·0 to 75·0μ	37·2μ	20·7 to 38·0μ
,,	V	17·6 to 27·0μ	18·7μ	18·5 to 23·0μ
,,	VI	3·3 to 7·7μ	3·8μ	1·6 to 2·7μ
Total length of parasite		102·6 to 134·0μ	76·7μ	59·5 to 87·0μ
Width of nucleus		3·2 to 5·7μ	2·2μ	1·6 to 2·7μ

V.—The remaining trypanosome to be described is a long narrow form, with a narrow nucleus, and a large blepharoplast (Plate XXVI, fig. 24). It measures as follows:—

Measurement	I	... ... 3·3 to 6·6μ
,,	II	... ... 13·2 to 19·2μ
,,	III	... ... 2·2 to 3·3μ
,,	IV	... ... 6·6μ
,,	V	... ... 11·0 to 16·0μ
,,	VI	... ... 1·1μ
Total length of parasite		... 37·0 to 54·0μ

The cytoplasm is rather coarsely reticular, the body is pointed at both extremities, the large blepharoplast is situated in the centre of a clear space, from which arises the rather scanty undulating membrane.

This parasite resembles *T. inopinatum* in many ways, and may be a parasite of that form.

The question arises whether the cycle of multiplication just described is completed in the frog, or whether it normally occurs only outside the frog, probably in a second blood-sucking host, as a leech. The smallest rounded parasites with flagella and the herpetomonas-like forms never occurred in freshly-drawn blood. Bouet[6] agrees with this observation in stating that none of the young parasites were seen in the blood. Franca and Athias,[6] however, record that they saw small rounded parasites both with and without flagella (probably forms of *T. loricatum*) in blood fixed immediately after withdrawal. A single small rounded parasite (6μ in diameter) was seen in our series of slides of freshly-drawn blood. With the exception noted above, every other type of parasite occurring in the developmental cycle described in this paper was observed in fresh-drawn blood. From these observations it seems that this cycle of "swarm" division may be completed in the amphibian host, but that the smallest forms are rarely present in the peripheral blood. It is noteworthy in this connection that there is no certain correspondence between the length of time blood containing *T. loricatum* has been kept and the type of trypanosomes present in it.

More than once large specimens of *T. loricatum* were seen in whose substance red blood cells occurred. Marchoux and Salimbeni believe that the cells and the parasite are merely superimposed. Without wishing to assert that the cell has been ingested, that is, that its presence in the parasite is due to more than a mechanical accident, we are confident that we have seen instances where the cell was definitely within the cytoplasm of the trypanosome. The trypanosomes may be attacked at any stage of their development by leucocytes. Frequently they seem to resist successfully and are not ingested. In one instance a leucocyte was seen to ingest the haemoglobin containing stroma of a disintegrated red cell; it was interesting to note that its protoplasm contained dark brown granules —a most unusual occurrence.

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*Drepanidia.**

Fresh Preparations.

Drepanidia were present in every species of frog and toad named on page 307, and in almost every frog examined. In the same frog (*Rana galamensis*) in which the developmental changes in *T. loricatum* were observed, drepanidia were present in almost every other red blood corpuscle, though scarcely any young parasites were seen. The red cells were swollen to nearly twice their normal size, the colouring matter had disappeared, and the cells in fresh preparations presented the appearance of a crumpled colourless bag, to one side of which the parasite lay. The nucleus was pushed to the periphery of the cell (Plate XXIX, fig. 86).

A few of these drepanidia were found free in fresh preparations examined immediately after making them, but the number of free parasites increased enormously after half an hour. The drepanidia, after leaving the cells and becoming free, were long and had a round anterior end, *i.e.*, the end in advance during progression (Plate XXVIII, fig. 67). A little way from this end the body tapered gradually to the posterior end, which though narrow, was bluntly rounded off (Plate XXVIII, figs. 68 and 69).

Progression takes place in three ways:—

(1) With the body stretched out by a gliding movement.

(2) By gregarine-like contractions of the protoplasm. This occurs when obstruction is encountered (Plate XXVIII, figs. 70 and 71).

(3) By a sudden jerking backward of the posterior end which has previously curved round to meet the anterior end.

* See footnote to page 303.

After about an hour, agglutination of the free parasites was observed. They joined themselves together by their posterior, sharper, somewhat granular ends. Two, four, or six parasites came together in clumps in this way. Contractions occurred from side to side, causing the parasites to bend. Two days after the fresh preparations were made, the agglutinated groups of drepanidia were observed still, the individuals being rather granular and stumpy and actively motile, but they did not undergo any further change.

Three forms of drepanidia were observed, a small form (Plate XXVIII, fig. 74) which will be described in stained preparations, a medium form (Plates XXVIII and XXIX, figs. 82, 68, 69) (the common form), and a very large form (Plate XXIX, fig. 86). These large drepanidia often contained large yellowish, highly refractile granules, which varied in size and number in different parasites. There were from one to about one hundred of these globules in each parasite (Plate XXIX, figs. 84, 85, 89, 90, 91, 92). These highly refractile granules were situated for the most part around the nucleus. In some of these parasites small dancing granules were seen at the more slender end.

Stained Specimens.

The following description is based on the examination of dried and stained films.

The nucleus of the host-cell was displaced (Plate XXVIII, fig. 59) but not injured; the cytoplasm, however, suffers severely since the parasite seems literally to tunnel about within the limits of the cell. Many corpuscles were found in which the contents seemed to have been devoured, leaving them looking like wrinkled empty sacks (Plate XXVIII, fig. 67). Such sacks could be seen with the drepanidia present, or leaving, or gone. Two and three parasites were sometimes seen in one blood corpuscle. The drepanidia were not only present in erythrocytes but were also occasionally found in leucocytes.

As already noted there were three principal forms of drepanidia; a large form, a medium form, and a slender form. The medium form was the most common (Plate XXIX, figs. 81, 82), and was from 15 to 18μ long and from 5 to 6μ wide. It had a fine granular striated protoplasm, which sometimes contained coarse red granules, and sometimes not. Some specimens had a few (four) granules at the anterior end of the body, and some had a straight blue line running

from the nucleus to the anterior extremity. The nucleus was from 3·3 to 4·4μ in diameter, and was placed about 7μ from the anterior extremity and 4μ from the posterior extremity.

One and two constrictions in these forms were observed, showing the method of progression described in fresh specimens (Plate XXVIII, figs. 70, 71). In a few specimens the nucleus had apparently divided into two, and the halves were attached by a red line (Plate XXVIII, figs. 68, 69).

Some of these forms were encysted, both in erythrocytes and free in the serum. Such encysted forms often had a small amount of red-staining excretion at both ends (Plate XXVIII, figs. 59, 60).

The "slender" forms (Plate XXVIII, figs. 73, 74) were from 13 to 15μ long and 1·1μ wide. The cytoplasm was light-staining and of a loose texture. The shorter or posterior end was very faint-staining and the extremity could be seen with difficulty. The longer or anterior end was a faint pink, which deepened towards the extremity. On both sides of the nucleus were areas staining the same as the cytoplasm of the erythrocytes. There were two, sometimes three, of these areas which were from 0·6 to 1·7μ long. Near the posterior extremity there were sometimes from two to four or more red chromatin granules, and occasionally there were a few in the anterior part of the body. The nucleus consisted of eight peripherally arranged chromatic granules, "chromosomes,"* and one or two darker, central chromatic granules, "karyosomes," all connected by fine lines. In one instance (Plate XXVIII, fig. 73) a blue spiral line was found to run from one of the chromatic granules to the anterior extremity of the body. The nucleus was 2·2μ long and was placed 9μ from the anterior extremity, and 3·3μ from the posterior extremity. This "slender" form may be present in the blood with the larger form, or it may be present in blood which has none of the larger forms.

These "slender" drepanidia penetrate the erythrocytes, then lose both ends, so that only the nucleus and a small amount of cytoplasm around it is left. The chromatic granules go to the side, and later they are gathered in a mass at one end of the body, with a few chromatic granules remaining outside the mass (Plate XXIX, fig. 76). The body of the parasite enlarges and at the extremity opposite the chromatin mass are several round clear spaces, which may or may

* It must be understood that the terms chromosomes and karyosomes are used in a purely descriptive sense.

not contain a granule of chromatin. The mass of chromatin divides into two, and the cytoplasm may or may not divide synchronously. In cases where the cytoplasm does not divide synchronously, the chromatin goes on dividing until there are from 10 to 16 masses of chromatin (Plate XXIX, figs. 78-80) arranged almost around the edge of the cell. These rosettes later show divisions into young parasites (Plate XXIX, fig. 79). Rosettes were seen leaving the host-cell, and also found free in the serum (Plate XXVIII, fig. 66). In one slide a group of eleven young drepanidia that had just escaped from the rosette condition was seen (Plate XXVIII, fig. 72). These forms were 7·7μ long and 1·1μ wide. Their cytoplasm was of a faint-staining, coarse, granular structure. The nucleus was composed of a larger, central mass of granules and, usually, a chromatin granule on either side; occasionally both were on one side. In one case the chromatin seemed to be dividing (Plate XXVIII, fig. 72). These sporulating* forms always arose from the "slender" drepanidia. In one series of eight slides from one frog many sporulating and "slender" forms were seen, but none of the larger drepanidia described in the next paragraph.

In a few frogs, *Rana mascariensis*, a very large drepanidium was seen, which was like the common form, but larger. It was lighter stained and often took a pink colour instead of the normal blue. The cytoplasm was coarsely granular, and many of the parasites had non-staining, highly-refractile globules in them. These globules were from one to one hundred in number, and from 0·6μ to 7·7μ in diameter. They were always found near the nucleus and gave the appearance of a fatty degeneration of the nucleus. They sometimes distended the drepanidium until it was nearly round (Plate XXIX, fig. 89), but they usually did not affect the size of the parasite. The large type of drepanidia presented three forms, a long form, a short or young form, and a folded or "two-shanked" form. The long form (Plate XXIX, fig. 86) was usually 22·6μ long and 7μ wide. The nucleus was 3·4μ long and 5·6μ from the posterior extremity and 13·6μ from the anterior extremity.

The short, or young form (Plate XXIX, fig. 83) was about 8·8μ long and 6·6μ wide. The nucleus was 4·4μ in diameter and placed in the centre of the organism.

The folded form was seen in the various stages of unfolding (Plate XXIX, figs. 87, 88).

* The term is not used in its specific sense.

Cytamoeba.

Cytamoebae were present in a few of the frogs in considerable numbers. Amongst the forms seen was the round form (Plate XXIX, figs. 104, 106, 108, 110) previously described in the snake (page 304) (2·2μ wide). In it were short rods, or sometimes round, red dots (Plate XXIX, fig. 104). Other forms were oval or finely granular, sometimes with no apparent internal structure (Plate XXIX, fig. 103). The round forms were often found near the nucleus, and sometimes apparently emerging from it, giving the appearance of fragmentation of the nucleus (Plate XXIX, fig. 110). Some of the parasites were filled with slender rods, which at times projected beyond their edge for a distance of 4μ (Plate XXIX, figs. 112, 113). Other forms were present which looked like masses of rods bound together (Plate XXIX, figs. 107, 108, 109, 115); sometimes these bundles of rods were free in the serum (Plate XXIX, fig. 100). Occasionally short rods were found free in the erythrocytes, sometimes end to end, sometimes crossing one another at right angles. The rods found in all the above forms were of two kinds, a short rod with rounded ends, and a long filamentous rod. Some of the short rods were seen free in the blood plasma in such forms and arrangements that there seems to be no doubt that some of them at least are bacteria. It is equally certain that some of the longer slender rods are merely filamentous cytoplasmic processes of the rounded parasite. In some of the specimens the body of the cell was stained a light blue, while the rods within were stained a bright red.

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An unidentified parasite

Another parasite, a round, red-staining mass from 1·5 to 5·5μ in diameter, is found in red cells (Plate XXIX, figs. 93, 95, 99). With it are associated bluish-green, crystal-like bodies (Plate XXIX, figs. 93, 95, 97, 98). Both the red masses and crystals may be present in varying numbers (Plate XXIX, figs. 98, 99). The cytoplasm of the host-cells is usually stained a deeper blue than usual. Many of the smaller red masses have a well-defined blue area around them, which suggests that this parasite possesses a cytoplasmic body (Plate XXIX, figs. 94, 96). In stained films of fresh blood the red masses do not seem to have much structure, but in films of blood kept for two days, the structure consists of chromatic granules arranged peripherally with one or two darker chromatic granules in the centre.

This parasite with its crystals was seen in fresh specimens. It occurred in conjunction with drepanidia and *T. loricatum.*

FISHES*

Fresh-water fish of many different sorts were examined in the Gambia and in the Congo. Parasites were found only in the fish without scales, mentioned below.

Those infected were caught in a very small sluggish stream with a very dirty bottom; none of them were over seven inches in length and none were infected with drepanidia.

Trypanosomes (Plate XXX).

Two "mudfish" (*Clarias angolensis*) caught at Leopoldville in the Congo Free State on December 9th and December 30th, 1903, had actively motile trypanosomes in their blood which may be divided into three types; a small, a medium, and a long form. The small type was identical in both, the medium was present in both, but with slight differences, and the long form was present only in the fish of December 9th. The small form was very frequently seen in the fish of December 30th, while in the fish of December 9th, only four were present in a total of ninety-nine parasites. The medium form, in the fish of December 30th, was less frequently met with than the small form, but was often seen in the fish of December 9th. Of the long forms only thirteen were seen. All the forms were characterised by the possession of a large four-lobed blepharoplast,

* The description of the parasites found in fishes is republished by permission from the Journal of Medical Research.

situated at, or very near, the posterior extremity, and by a clear space about the nucleus. The blepharoplast apparently consisted of four darkly-staining granules of equal size embedded in a matrix. In a few specimens there were, perhaps, more than four granules in the blepharoplast, but in these cases it was never possible to be certain of the exact number. The three forms of parasites seemed but different variations of one species, as gradations between all forms could be seen.

The small type was readily distinguished by its size, its narrow body, its relatively long nucleus—situated more anteriorly than in the other two types, and with its long diameter parallel to that of the body—and by its faint-staining reaction. The blepharoplast was oval, or round, large and distinct (measuring 1·13 by 0·75μ), and was situated at the posterior extremity. The undulating membrane was well developed, having a width of 0·75μ. The long, oval nucleus was situated at the junction of the middle and anterior thirds of the body. It was granular in structure and contained a fibrous network on which were seen eight to sixteen chromatin granules, and one darker granule—the karyosome. The body protoplasm was reticular, and contained a few violet-staining granules. Occasionally parasites were seen in which the body was filled with these coarse chromatophilic granules. Superficial longitudinal striations to the number of five could be seen near the nucleus of two slightly injured parasites. Division forms were very numerous in this type (even when but four were seen in the fish of December 9th, one of them was dividing). They followed the usual method of longitudinal division. In the division of the blepharoplast, the posterior lobe on the concave side of the parasite moved behind the other posterior lobe; the axis of the posterior lobes thus became longitudinal, while that of the anterior lobes remained transverse. The two posterior lobes then separated from the anterior lobes and their axis became transverse again.

The medium type was characterised by its size, and its darker staining reaction. The blepharoplast was large, oval or round, distinct and measured about 1·5 by 0·75μ. It was situated at the posterior extremity, or from 1 to 1·5μ anterior to it. The flagellum was relatively longer than in the short form. The undulating membrane in parasites from the fish of December 30th was well

developed, and wound in numerous folds around the body; it was 1·5 μ wide. In trypanosomes from the fish of December 9th, the undulating membrane was poorly developed, had a few folds and was not more than 1 μ in width. The nucleus was round to oval, its long diameter was at right angles to that of the body, and it occupied the whole width of the parasites. It was granular in structure and contained a fibrous network on which were seen sixteen to twenty-four chromatin granules and one or two darker granules—the karyosomes. The body was more darkly stained in the fish of December 30th. Superficial longitudinal striations to the number of seven were counted near the nucleus. Divisional forms were few in this type and followed the usual method of longitudinal division.

The long type was characterised by its size, its tapering extremities, its comparatively short flagellum and by the possession of from one to four vacuoles just anterior to the blepharoplast. The blepharoplast was large, oval or round, and situated 2 μ from the posterior extremity; its measurements were the same as those of the medium type. The nucleus had the same characteristics as that of the middle type, except that it did not quite occupy the width of the body. The protoplasm was the same as in the medium type, except for the above mentioned vacuoles, which were always present. Eight superficial longitudinal striations were present and these were seen to cross the nucleus. No divisional forms of this type were seen.

The measurements of the three types were as follows:—

	Small form	Medium form	Long form
Measurement I	... 0·0 μ	... 0·0 to 1·5 μ	... 2·0 μ
,, II	... 12·7 μ	... 14·2 μ	... 25·0 μ
,, III	... 3·0 μ	... 3·7 μ	... 45·0 μ
,, IV	... 6·7 μ	... 12·0 μ	... 19·5 μ
,, V	... 12·0 μ	... 13·5 μ	... 10·5 μ
,, VI	... 1·0 μ	... 3·0 μ	... 4·5 μ
Total length ...	... 34·5 μ	... 45·0 μ	... 61·5 μ

Although the trypanosome described by Montel[1] closely resembles our large form, we have seen no account in the literature of any parasite morphologically identical with those described above.

LITERATURE

Good lists of the publications on the trypanosomata of fishes will be found in the works of Laveran and Mesnil, "Trypanosomes et Trypanosomiasis," and of Mense, "Handbuch der Tropenkrankheiten."

The following are important papers not mentioned in these volumes:—

1. MONTEL. Trypanosoma d'un poisson de Cochinchine. Comptes Rendus de la Société de Biologie, T. I, 1905, p. 1016.
2. LEBAILLY. Recherches sur les Hématozoaires des Téléostéens marins. Thèse Fac. Med. Paris, 1906, p. 76; and Archiv. de Parasitologie, T. X, 1906.
3. LEBAILLY, M.C. *Trypanosoma bothi* (Barbue) n. sp. Comptes Rendus de la Société de Biologie, T. IX, 1905, p. 304.
4. BRUMPT. Sur quelques espèces nouvelles de Trypanosomes parasites des Poissons d'eau douce. Comptes Rendus de la Société Biologie, T. LX, 27th Jan., 1906, page 160.
5. BRUMPT. Mode de transmission et évolution des Trypanosomes parasites des poissons. Comptes Rendus de la Société de Biologie, 17th Jan., 1906, T. LX. No. 4, p. 162.
6. BALFOUR. Second Report of the Wellcome Research Laboratories, Khartoum, page 197.

A Spirochæte (Plate XXXI).

In the blood of the fish of December 30th a spirochaete-like organism was found which could be identified with no previously described spirochaete.

It had a hair-like body; its length was usually 18μ; its breadth at the widest part was 0·6μ. It was widest at one extremity or towards the middle; in the latter case, the parasite tapered towards both ends, but one extremity was always much thicker than the other. The wider end was rounded, while the other end was drawn out to a fine and slender point. The parasite usually occurred singly and lay in a simple curve, forming three-quarters of the circumference of a circle (Plate XXXI, figs. 1 and 2). Other forms—coils (fig. 3), compound curves (figs. 4, 9-12) and spirals (figs. 5-8)—were seen as illustrated. The coils were always formed by the slender end of the parasite. The spiral forms were long, measuring from 24-27μ, and in some of these forms there was a suggestion of a membrane (figs. 5 and 6). The parasites were also seen in pairs and in groups of from three to many individuals as shown in figures 13, 14-16. These organisms seemed to consist of a deeply-staining core and a lightly-staining periplast. Many of them stained irregularly by modifications of Romanowsky's method. The lighter stained areas (fig. 2) occurred at irregular intervals and were of

irregular extent. The periplast could be seen at these lightly stained areas and it was continued to form the slender extremity from which the core was also absent. Occasionally granular forms were seen in which the protoplasm of the core apparently arranged itself into small granular masses, but no regularity could be detected in the arrangement of these granules. Undoubted multiplication forms were not seen.

For descriptive purposes we propose for this organism the name of *Spirochæta jonesii*.

ARTHROPODA

The protozoa found in this class were encountered by chance during an investigation of the development of various haematozoa, while examining various blood-suckers, either as controls, or for the presence of developmental forms of the blood parasites. Many tsetse flies and mosquitoes, a few *Stomoxys*, ticks of various sorts, fleas and lice, and "Congo floor maggots," were examined. The only protozoan parasites observed are recorded below.

MOSQUITOES

(1) *Myzorhynchus paludis.*

A cluster of radiating club-shaped bodies (Colonie radiée) was seen in the thoracic tissues of a female of the above species, examined for malaria at Lusambo. The parasites became free, developed a flagellum and were actively motile in the salt solution in which the mosquito was dissected.

(2) *Pyretophorus costalis.*

Some excitement was at first created during the examination of the alimentary canal of mosquitoes, fed at varying antecedent periods on animals infected with trypanosomes, by the presence of vermicule-like bodies (Plate XXXII, fig. 14), measuring about 18 by 2·2μ. These parasites progressed slowly by active lashing and slower amoeboid movement; some similar forms were motionless.

The amoeboid movements were of two sorts. The first involved a change in the whole shape of the parasite. The second was by a simple protrusion of protoplasm from the rounded anterior, never from the effilated posterior extremity.

They occurred either within the stomach, in the stomachal tissues, or in the body cavity of the mosquito. A central differentiated area could be detected in them by examination in fresh specimens; near the area were more or less numerous granules which often exhibited lively Brownian movement. After some time the parasites became much stumpier (8μ by 16μ), and were later almost spherical. One parasite, however, remained unchanged and active for twenty-four hours.

Further search showed that the parasite occurred in freshly-caught and freshly-hatched mosquitoes, and in the larvae from which all our experimental mosquitoes were raised, and it was quickly shown to be a stage in the life cycle of a coccidium. It was most interesting that practically every larva from one pool was infected with this coccidium, while none of the larvae and adults (anopheline and culicine) from a pool only a few yards distant were affected. So far as was observed the parasite did not occasion any excessive mortality amongst the mosquitoes infected. This seems difficult to understand—the mosquitoes were probably not watched long enough—since the tissues of many of them were fairly riddled with coccidium cysts. The water of the heavily-infected pool was centrifugalised and examined. Coccidium cysts were not recognised, various infusoria and a clump of herpetomonas-like flagellates were alone seen.

So soon as it was certain that the vermicule-like "sporozoite" had no connection with the trypanosomes ingested, the study of this coccidium was discontinued. It is, therefore, not possible to say whether the forms observed represent stages in more than one parasite or no. It seems probable that but one species of coccidium was present. We describe the forms observed and indicate the position they seem to occupy in the life cycle of the parasite. The mobile sporozoites first seen become free through the rupture of a sporoblast containing eight sporozoites. The number of sporozoites in each sporoblast is almost invariable. Fig. 15 is unusual in that the nucleus of two of the sporozoites has divided, and in one instance division of the cytoplasm seems to have commenced. As a rule the long diameters of the sporozoites are parallel. These are, therefore, regularly arranged like the segments of an orange (corps en barillet). Single sporoblasts surrounded by a definite cyst wall may occur. They are more usually seen in groups of four, seven, or even as many

as eleven sporoblasts packed together in an oocyst with a definite capsule. The individual sporozoites have no capsule. Their cytoplasm stains a light blue by Romanowsky's method. It is alveolar in structure and rarely contains granules of any sort, but may have occasional granules. The nucleus is placed centrally, is loose in texture and consists of a varying number of chromatin granules placed in a more lightly-staining matrix. From the analogy of other coccidia, we assume that the forms just described represent the completion of sporogony. The male and female gametes, which probably commence this cycle, and their conjugation have not been seen. Neither has the further development of the sporozoites been observed.

The number of rounded merozoites (figs. 16, 17, 18), present in every part of the larvae and adults dissected, was often extraordinary. These organisms measured about 4 to 5μ in diameter. They were either free or enclosed in a host cell. On becoming extracellular they were seen to possess a definite capsule (pink-staining); this was quickly discarded (figs. 18, 19) and the empty shells were very frequently seen. Often merozoites which had just cast their capsules occurred in groups of four (fig. 18). Four merozoites were, however, never seen within an unbroken capsule. As a rule whether intra or extra-corpuscular the merozoites occurred singly. When extra-corpuscular they were occasionally in pairs. Sometimes large intra-corpuscular cysts were seen which contained very large numbers of merozoites. The merozoites (figs. 16, 17, 18) consisted of a pinkish alveolar cytoplasm in which was situated a nucleus usually surrounded by an indefinite bluish area. In this bluish area a lighter, rounded spot was almost invariably present. A few chromatophilic granules and small clear spaces were frequently seen in the cytoplasm. The nucleus consisted of irregular masses of chromatin placed in a matrix. In some forms (fig. 16) the granules were placed at the periphery of the nucleus and were joined by threads with a central darker-staining mass. In fresh specimens they were immobile.

LITERATURE

1. Ross. Parasites of Mosquitoes. Jour. of Hygiene, Vol. VI, No. 2, April 1906. The subject is reviewed and discussed.

TSETSE FLIES (*Glossina palpalis*)

Parasites of the type described below were found in specimens of *Glossina palpalis* freshly-caught, and at all periods, up to 11 days, after having fed on known animals infected with trypanosomes. All the parasites were not precisely similar, but one description will suffice for all. The free rod-like parasites were found only in the alimentary canal, nearly all of them in its anterior part. They were most numerous near the oesophagus, in the "stomach" or in the neighbouring part of the intestine. Cysts containing them were seen attached to, or actually in, the wall of the intestine. Some of them were whole, others had burst (or were burst by slight pressure), and from them were expelled the rod-like bodies. In fresh preparations these rods (Plate XXXII, figs. 1, 2, 3, 4, 5, 8, 9, 10) were non-motile, they contained one or two refractile granules and they occurred singly or, occasionally, in small clumps. The most usual form seen in stained specimens was a rod about 7·0μ in length and 1·0μ in width with abruptly rounded ends (figs. 1, 2, 5). It was limited by a definite outline, but apparently not by a capsule. Its body substance consisted of pale blue protoplasm in which occurred chromatophilic granules and rounded clear areas. The chromatophilic granules varied in size from extremely fine particles (fig. 6) up to masses measuring about 0·3 to 0·5μ in width (fig. 3). They were usually distributed more or less regularly in transverse bands (figs. 1, 2, 5, 10) lying, at intervals, across the parasite. In such parasites the individual granules could barely be distinguished. Often between each band of granules was placed one of the rounded, clear areas (figs. 1, 2, 5, 10). Such parasites were probably preparing for transverse division, as small forms, apparently consisting of one or two rounded segments of the original rod-like body, were often observed (fig. 7). In some of the parasites showing the band-like distribution of chromatin, as well as in others in which pairs of granules were arranged in bilateral symmetry (figs. 8, 9), it seemed as though longitudinal division might be about to occur. In many parasites the chromatin granules were distributed absolutely without order (figs. 3, 4, 6). Rarely a larger and a smaller granule seemed to be in connection with each other.

Rod-shaped parasites of all these three types were frequently terminated by a small chromatophilic granule (figs. 4, 5, 10) (sometimes placed in a clear area), or by a rounded enlargement which

often measured twice the diameter of the rest of the parasite (fig. 10). Rounded parasites measuring from 3μ to 5μ or more in diameter with irregularly distributed granules and no clear areas occurred (figs. 11 and 13). The smaller rounded forms can divide transversely; apparently after a concentration of the chromatin (fig. 12).

It seemed possible that this parasite should be placed amongst the *Myxosporidia*. In recognition of the collaboration of Dr. Inge Heiberg in the work of our expedition, and in particular of his observations on *T. loricatum*, we suggest for the name of this parasite *Myxosporidium heibergi*. About half of the tsetses dissected at Leopoldville had these parasites free in their midgut. In the stomach wall of one freshly caught fly coccidian-like cysts were seen. (" Corps en barillet " ; compare the parasite of *Pyretophorus costalis* described above).*

EPILOGUE

Although the facts presented in this paper are admittedly the results of passing observations, incomplete, disconnected, and made under unfavourable circumstances, we think that they possess a certain value as a record alone of protozoan infections encountered by chance during three years' work in Africa. Many of the appearances noted, though incomplete in themselves, are of additional value in that they confirm the constancy of morphological changes noted in other allied and better studied parasites.

The following examples are cited. Longitudinal striations have been seen in every trypanosome examined; the myonemes of other authors are, therefore, constant in the trypanosomes we have dealt with. In several instances the thickened edge of the undulating membrane of trypanosomes has been observed to terminate, not in the blepharoplast, but in a lighter-staining pinkish granule, or pair of granules, closely applied to it. In some of our trypanosomes the structure of the nucleus and the occurrence, in neighbouring differentiated areas, of paired chromatophilic granules suggests a connection with the complicated nuclear developmental phenomena observed in trypanosomes by some observers. The clear, unstained

* This is the last of the parasitic protozoa observed during our expedition to the Gambia and the Congo. The filariae found in various mammals, birds, amphibians and reptiles will be considered in a forthcoming paper by Professor. H. E. Annett.

perinuclear area, the irregular, non-staining almost granular areas, and the lines in the cytoplasm as well as the chromatophilic granules (see Plate XXVII, fig. 34) frequently observed in various trypanosomes described in this paper, are all constant phenomena, which seem to be of biological significance. In the development of *T. loricatum* the work of Danielewsky was confirmed and extended to prove a most interesting cycle of multiplication in a well-known trypanosome; it must be asked whether a similar cycle may not occur in other trypanosomes. The rough resemblances between the spirochaetes seen in ulcers and in fish with previously described parasites of this nature are striking. In the leucocytozoon of birds an interesting course of development is described.* Unfortunately our material was too scanty to permit us to fully compare our observations with those of Schaudinn. Very little has been said concerning the drepanidia observed. In spite of a very careful search in kept preparations of blood, we were unable to find any indication of a direct relation between this parasite and the trypanosomes which were often associated with it.† Neither were parasites resembling *Trypanosoma inopinatum* seen to become intracorpuscular.‡ In both stained and fresh preparations of frogs' blood, however, the great resemblance in size and general appearance between some trypanosomes and some drepanidia was very apparent. It was also noticed that, as a rule, if there were many drepanidia in a given frog, there were also many trypanosomes.

One of the earliest of the lessons learned from our work on these protozoa was the entire inadequacy of the methods of preparing blood-films, ordinarily employed by pathologists, for a morphological study of protozoa.

The examination of dried films stained by modifications§ of Romanowsky's method suffices for the purposes of clinical diagnosis. This method will show many of the structures present, it was used in

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* The dangers of constructing a part of the life-history of a parasite from stained specimens alone are apparent. The course of development we described may be mistaken, but the descriptions of the forms mentioned are accurate and may be relied upon so far as the defects of the method of preparation employed permit.

† Billet, Culture d'un trypanosome de la grenouille chez une Hirudinée. C. R. Acad. des Sci., T. CXXXIX, Oct. 10, 1904, p. 643.

‡ Billet, *Trypanosoma inopinatum* et sa relation avec les Drepanidium. C. R. Soc. de Biol., July 23, 1904, p. 161.

§ Stephens and Christophers. The Practical Study of Malaria, page 10, University Press of Liverpool.

the whole of the work described in the present papers, but it fails to demonstrate finer details properly. Our work must, therefore, be regarded as incomplete, and certainly the more important parts of it should be repeated by workers using methods more perfect than those employed by us.

A second lesson was that too much of the work done on the pathogenic protozoa, particularly by medical men, has been directed by conceptions derived from bacteriology.

Days of tedious searching of slides and of observation of parasites, placed under various conditions, must be spent in studying the pathogenic protozoa, when minutes would almost suffice in the case of bacteria. The difficulty of finding the parasites at all is sometimes extraordinary, and the possible occurrence of a latent infection must never be forgotten.

The study of the pathogenic protozoa must be approached with an unbiassed mind and with the remembrance that the known life-cycles of several protozoa are exceedingly complicated. We believe that the continuous observation of living parasites will ultimately furnish the richest reward. A single positive observation so obtained is absolute, and outweighs any number of apparently antagonistic probabilities obtained by deductions from work done along apparently parallel lines of research. Of course, the examination of fresh preparations should be supplemented by the examination of stained material. Lastly, at the present moment more is known, in every way, of malaria than of almost any other disease. In observing less studied protozoan infections it will frequently happen that our knowledge of what actually does occur in malaria will lead to the formation of an ultimately successful working hypothesis, or to the correct interpretation of newly-observed phenomena.

EXPLANATION OF PLATES

All the illustrations accompanying this paper, except where it is otherwise stated, are drawn to a magnification of 2,000 diameters. No camera lucida was employed; the dimensions were obtained by measurement. A Zeiss 2mm. apochromatic 1·40 aperture objective, with an 8 or 18 eyepiece, was regularly used.

PLATE XX

Figs. 1 to 28, except 27, are "Young Forms"

As a rule, ectoplasm, endoplasm and nuclear material can be distinguished in each of these parasites; occasionally it is difficult to do so (fig. 24). The arrangement of the parasites and the host-cell are well seen in figs. 1, 25, 28. The constant presence of a clearer area about the chromatic material is to be noted in these parasites.

Fig. 1.—Chromatic mass with linear extension possibly antecedent to nuclear division, as in fig. 3.

Fig. 2—Shows nucleus with vacuole containing indistinct granules.

Fig. 3.—Nuclear material divided into a small and a larger chromatin mass connected by a line; possibly stage succeeding fig. 1.

Figs. 4, 5 and 9.—Chromatin divided into a larger mass and a smaller granular one, placed in a vacuole. Note the vacuole in these parasites is frequently pink-staining.

Figs. 6 and 7.—Forms similar to 2 and 5 in very small parasites.

Fig. 8.—Very small parasite showing a line.

Fig. 10.—Chromatin divided into two equal masses.

Figs. 11 and 12.—Are squashed and somewhat degenerated parasites. They are useful in that they show distinctly the presence of definite structure in the nuclear material.


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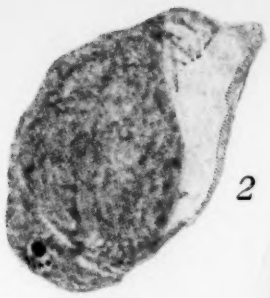
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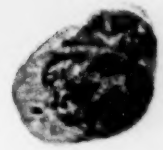
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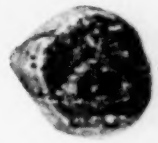
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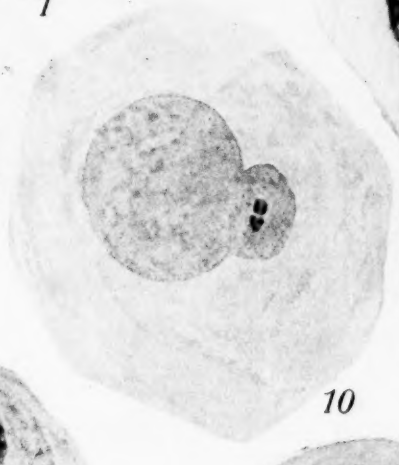
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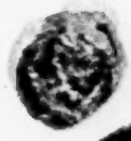
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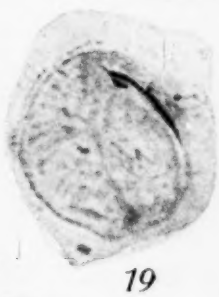
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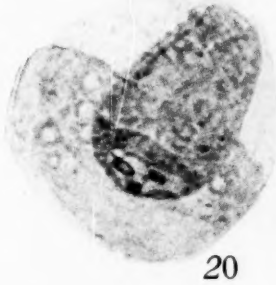
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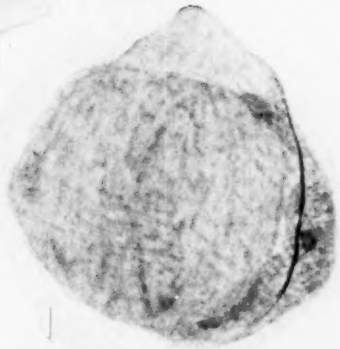
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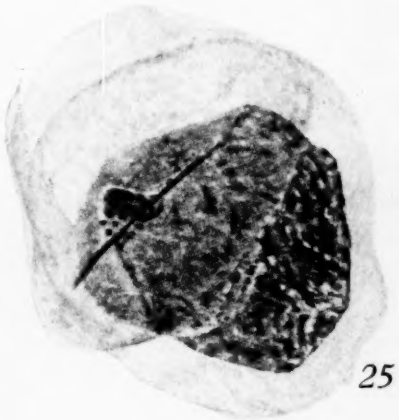
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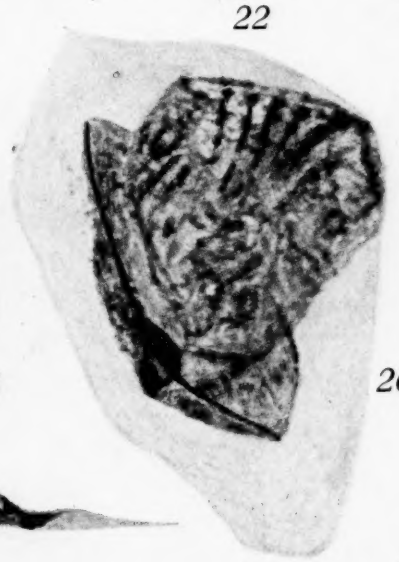
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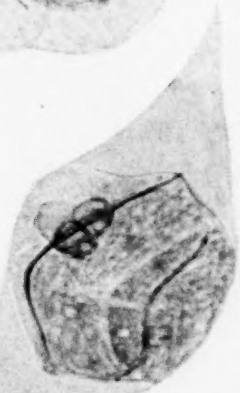
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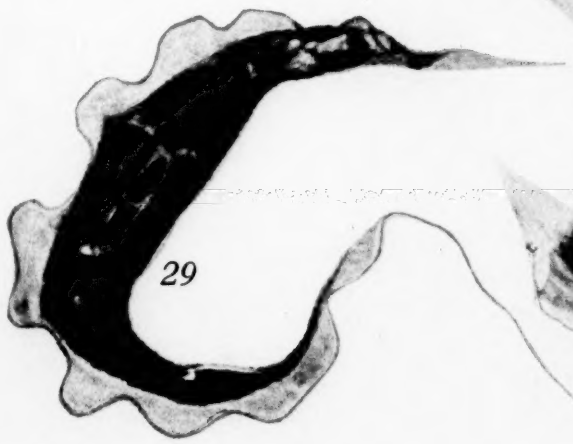
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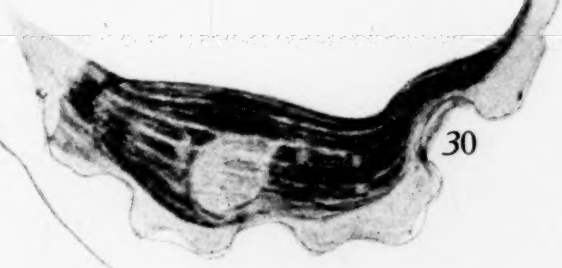
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PLATE XV

Fig. 13.—Shows a form in a small parasite probably comparable to 9. The vacuole is well marked. The endoplasm of the parasite has pressed upon and considerably distorted the nucleus of the host-cell.

Fig. 14.—Probably not similar to 1, but commencing formation of line.

Figs. 16, 17 and 18.—Early formation of line. Note its position in the chlamydoplasm.

Figs. 19, 20, 21 and 22.—Are unique specimens. They probably represent important stages of the maturation of the parasites. We feel it unwise to attempt to discuss their exact significance. In 22 a wavy bluish-line is disposed along "the line."

Fig. 15.—Is a squashed and degenerated parasite. The immediate connection of at least one pair of chromatophilic granules with the line is very evident in 15, 19 and 23.

Figs. 23, 24, 25 and 28.—Are well-grown parasites which retain their spherical form.

Fig. 26.—Shows commencing transverse division of the line. The connection of the "blepharoplast" with the blunt extremity of one half of the line is well shown.

Fig. 27.—Line shows commencing longitudinal division. Note that there are two distinct areas of chromatophilic material, beside the chlamydoplasm, lying immediately beneath the dividing line. (This specimen was unique.)

Figs. 29 and 30.—Trypanosomes.

Fig. 31.—Spherical female. Note dark granule connected with line running across chlamydoplasm; this is the only instance in which such a line was seen. The ectoplasm and host-cell nucleus are being thrown off.

Plate XXI

With the exception of fig. 38, probably a female, all the parasites illustrated in this plate are males.

Fig. 33.—Division of the line; nucleus only just commencing to divide.

Fig. 33a.—Division of line with chlamydoplasm and nucleus. The ectoplasm has been deleted for convenience of reproduction.

Fig. 34.— Formation of line (?). Note the presence of granules of chromatin.

Fig. 35.—A very common form (see figs. 42, 43, 74). It represents the line passing through a diffuse chromatic area (chlamydoplasm) in which lies a denser chromatic area (nucleus ?), often containing one or more (figs. 42, 41) deeply chromatic granules (blepharoplast ?). Occasionally these granules, or similar ones, lie outside the denser nuclear material (fig. 43).

Fig. 36.—Degenerated and squashed parasite, showing multiple nature of line.

Fig. 37.—Only three parasites of this type (all practically identical) were seen. It is remarkable in possessing a blepharoplast-like mass of chromatin distinct from the main nuclear structure. There is an indefinite arrangement of fibrillae and granules about the nucleus and chlamydoplasm.

Fig. 38.—A female cell (unique), in which a faint blue line was wound spirally about one half of the line. Appearances resembling this were seen in one or two other preparations (fig. 62).

Fig. 39.—Line dividing longitudinally, nucleus already completely divided. Parasites of a similar type are not infrequent. The one illustrated is considerably distorted.

Figs. 40 and 41.—Commencing transverse division of the line. In each parasite the line has become thinner. In fig. 41 the differentiated area surrounding the constriction is well seen.

Figs. 42, 43 and 74.—Are ordinary types of adult males with lines.

Figs. 41, 42 and 43.—Blepharoplastic (?) granules occur in the two former within, in the latter without, the nucleus. The presence in fig. 43 of a bluish area within the chlamydoplasm in immediate connection with the nucleus is suggestive.

Fig. 44.—The line is divided transversely (specimen unique); the two halves are connected by a filament.

Fig. 45.—(Compare figs. 63 and 64). Probably represents a stage anterior to fig. 46. (Specimen unique.)

Fig. 46.—The line is much involuted. It extends far into the ectoplasm. A faint blue spiral line (artefact ?) extends, from a double granule in the ectoplasm, through the lower part of the parasite. The arrangement of the nucleus is not at all definite.

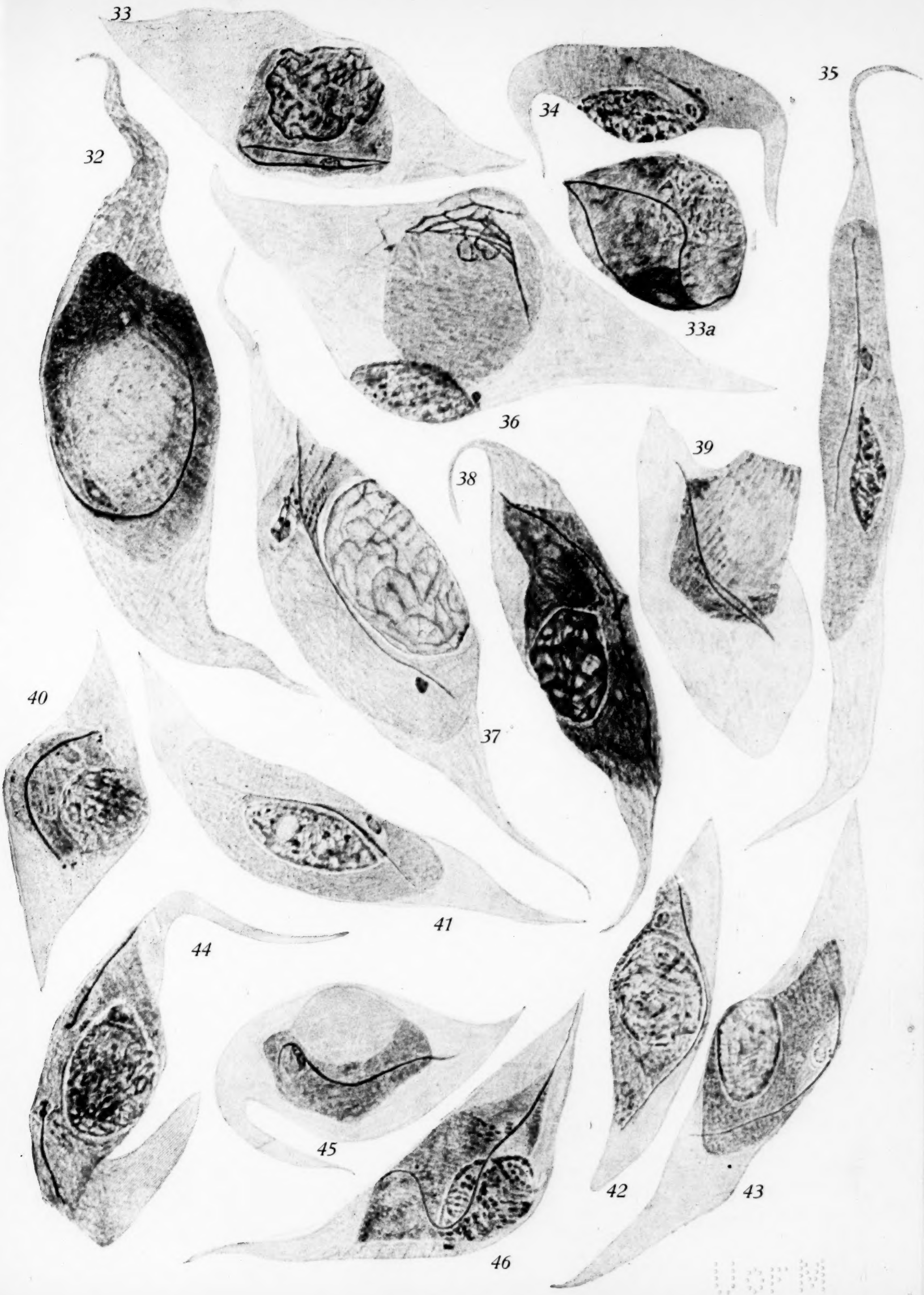


PLATE XXI.

Plate XXII

Fig. 47.—(Specimen unique. The colouring of this parasite is much too vivid.) Probably occurs at almost the same period as fig. 49. It is remarkable for the four granules placed in the concavity of the crescentic nucleus. (Similar dots have been seen in other parasites, but never again in this position).

Figs. 48, 49.—Are very common types of males. Note the spherical nucleus with its neighbouring granules in 48 and the distributed chlamydoplasm in fig. 49. (Compare fig. 75.)

Fig. 50.—Shows a disjointed bit of chlamydoplasm in relation with the blepharoplast. Note the granules in this position. The alveolar structure of the endoplasm has given the parasite a lattice-like appearance.

Figs. 51, 52, 53 and 55.—Are stages in the division of the nucleus. In fig. 53 the vacuole in association with the blepharoplast is well seen.

Figs. 54, 56 and 59.—The chlamydoplasm is abundant. The nucleus has formed a spindle-like figure, best seen in fig. 56. In fig. 59 the chromatic granules of the figure seem about to divide, and the vacuole in connection with the blepharoplast is well seen.

Fig. 57.—Two tiny granules are attached to the blepharoplast by fine threads. The nuclear chromatin is commencing to show polar concentration. (See fig. 60.)

Fig. 58.—Granules are being extruded (?) from both nucleus and blepharoplast.

Fig. 60.—Is probably the stage succeeding fig. 57. The nucleus has almost divided.

Fig. 61.—Shows enormous increase of chlamydoplasm. (Compare fig. 56.)

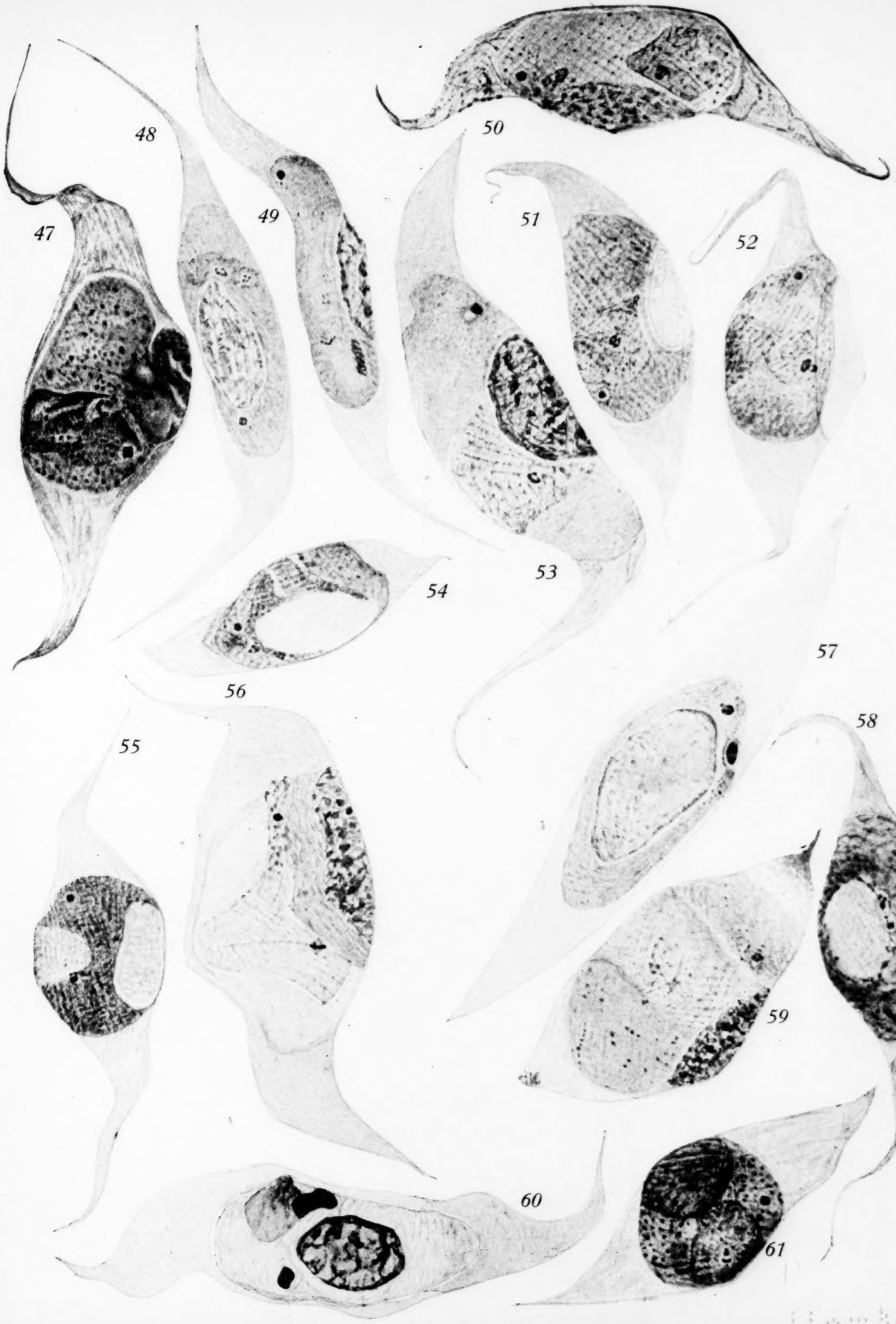


PLATE XXII.

PLATE XXIII

× 1,334.

Figs. 62-73, except 68, 70, and 71 are females.

Fig. 62.—Is a stage in line formation. Note the wavy blue line crossing the chlamydoplasm.

Figs. 63 and 64.—Compare fig. 45.

Fig. 65.—Note endoplasm lying over host-cell nucleus. Chlamydoplasm does not stain, so nucleus appears to lie in a vacuole.

Fig. 66.—Host-cell nucleus extruded, nucleus well differentiated. Note granules in chlamydoplasm.

Fig. 67.—Host-cell nucleus about to be extruded. Compact chlamydoplasm, well-defined nucleus (or blepharoplast?). This is a very common type.

Fig. 68.—(Compare figs. 48, 57, 71, 75.) Chlamydoplasm with spherical nuclear area. Blepharoplast very granular and one granule extruded. (Compare fig. 49.)

Fig. 69.—(Compare fig. 67.) Is a common type. The chlamydoplasm is very diffuse.

Fig. 70.—Parasite possessing distinctive characters of neither adult male nor female.

Fig. 71.—Note deeper-staining area in nucleus. (Compare fig. 68.)

Fig. 72.—Much effilated makrogametocyte; in one instance two fine chromatic granules occurred, as illustrated, in the chlamydoplasm.

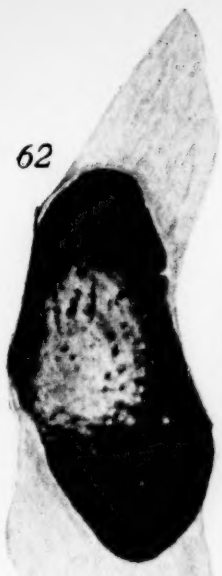
Fig. 73.—Is a common type of adult female; the vacuole in connection with the blepharoplast is well seen.

Fig. 74.—Compare fig. 35.

Fig. 75.—(Compare fig. 48, &c.) Note the two large ill-defined granules outside the nucleus in the chlamydoplasm.

Fig. 76.—(Compare fig. 49.) Is palely stained; chlamydoplasm is much extended.

Fig. 77.—Discarded ectoplasmic sheath and host-cell nucleus of an adult parasite.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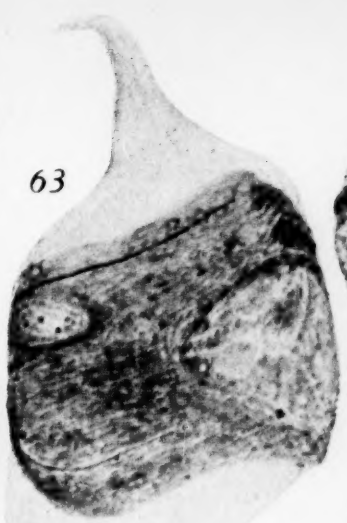
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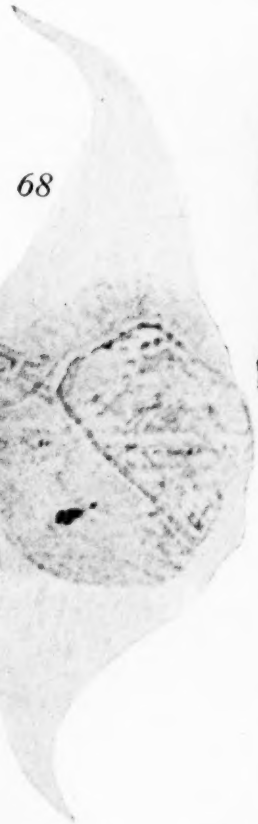
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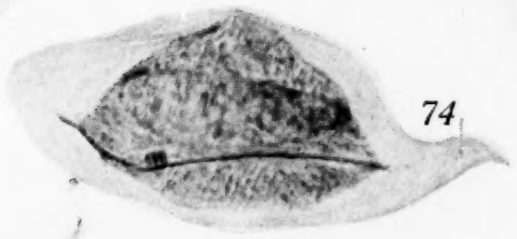
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PLATE XXIII

PLATE XXIV

Fig. 1.—"Slender" form.

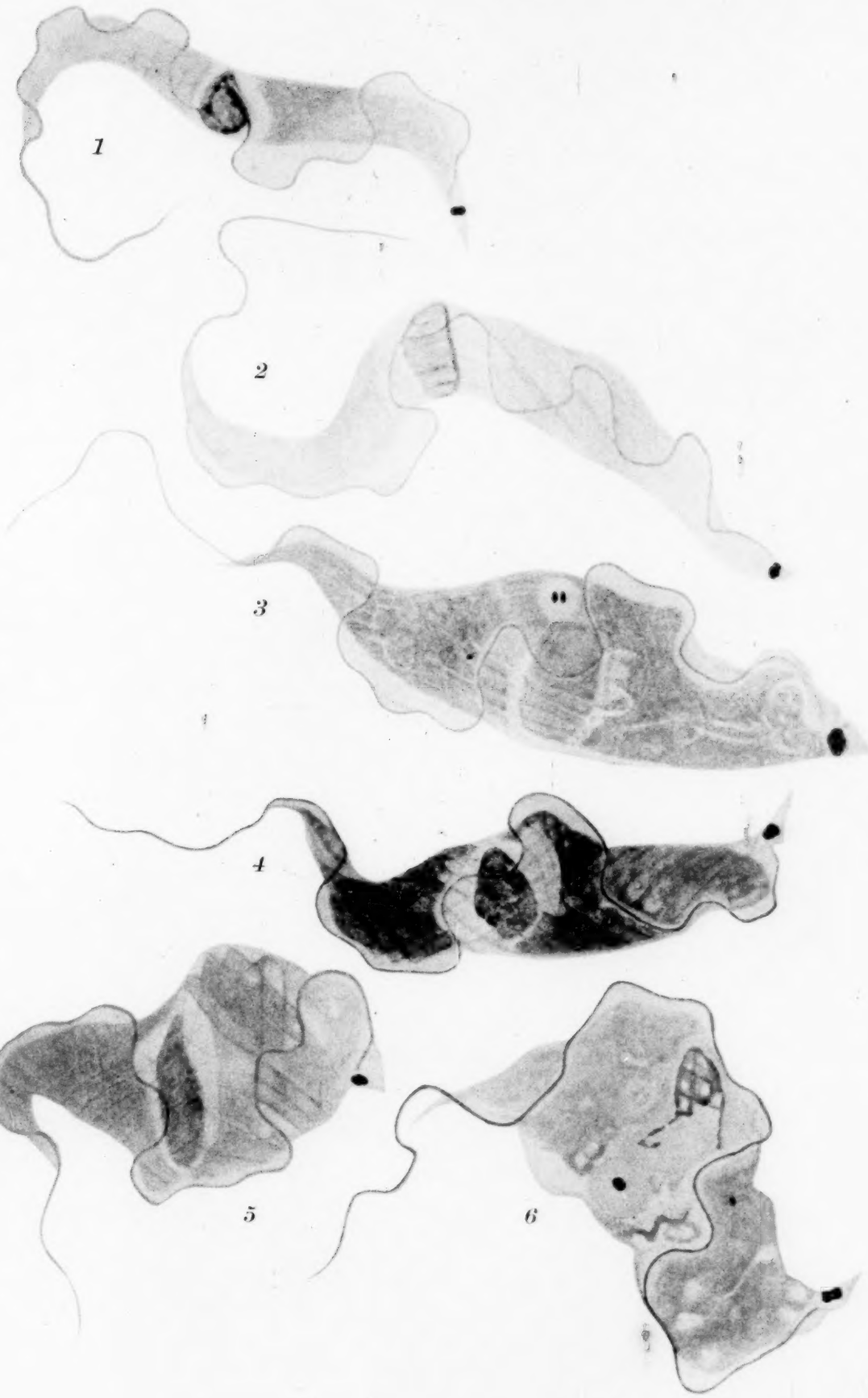
Fig. 2.—"Slender" form.

Fig. 3.—"Broad" form.

Fig. 4.—"Broad" form.

Fig. 5.—"Stumpy" form.

Fig. 6.—"Stumpy" form; evidently degenerated but reproduced to show structure of blepharoplast and nuclear granules.



Dutton, Todd and Tobey.

Trypanosomes.

PLATE XXIV

PLATE XXV*

Fig. 1.—*T. loricatum.* This is apparently an ordinary division form with two blepharoplasts, two nuclei and two flagella. The nuclei are long, sharply-defined sacs, with many small chromatin granules in them.

Fig. 2.—*T. loricatum* This form has a long nucleus with chromatin massed near both ends. There are longitudinal pleats and a short free undulating membrane.

Fig. 3.—*T. loricatum.* This form has a round nucleus. The longitudinal pleats are present.

Fig. 4.—*T. loricatum.* This form has deep longitudinal pleats. The nucleus is not so long as in figs. 1 and 2, but has the characteristic shape and curves towards the undulating membrane.

Fig. 5.—*T. loricatum.* Lanceolate form. The nucleus is a long and broad band. The undulating membrane runs as a white band in folds down the middle of the parasite.

Fig. 6.—*T. loricatum.* Long "hyla form" (page 324). The body has longitudinal folds. The nucleus is long, and wide near the middle, tapering to both extremities.

Fig. 7.—*T. loricatum.* Long "hyla form." The folds are unfolding. No nucleus is visible.

Fig. 8.—*T. loricatum.* Parasite has become rounded and is about to lose its flagellum.

Fig. 45.—Trypanosome of the tortoise, showing round unstained areas.

Fig. 46.—Drepanidia of the tortoise. The nucleus stains purple and both it and the cytoplasm are denser than in fig. 48.

Fig. 47.—Drepanidium of the tortoise. The nucleus is red and the body has many coarse granules in it.

Fig. 48.—Drepanidium of the tortoise. The nucleus is red and loosely woven, and the cytoplasm is also loosely woven.

Fig. 49.—Drepanidium of the crocodile.

Fig. 50.—Drepanidium of the snake—common form.

Fig. 51.—Drepanidium of the snake. The nucleus and cytoplasm are loosely woven, and vacuoles are present at both ends.

Fig. 52.—Drepanidium of the snake, showing elongation of the body of the host-cell.

Fig. 53.—A curious appearance in a snake's blood (page 304); free form, red dot in the middle.

Fig. 54.—The same in a cell with no drepanidium.

Fig. 55.—The unidentified parasite of the snake—round form.

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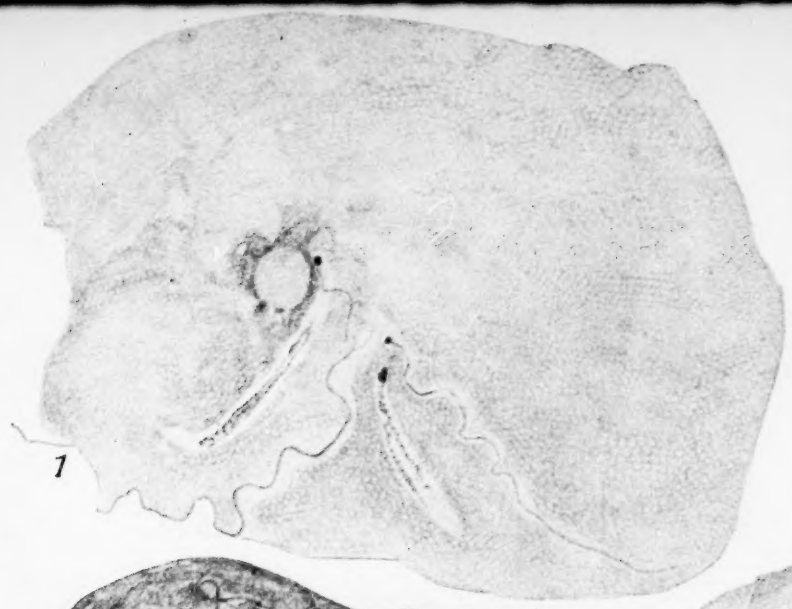
* Numbers 1-8 are drawn one-half the usual size.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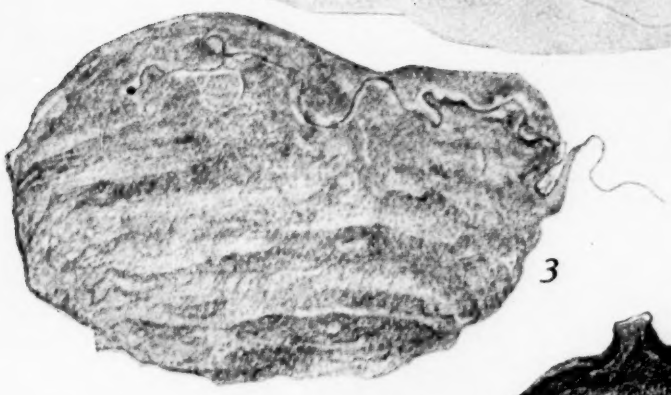
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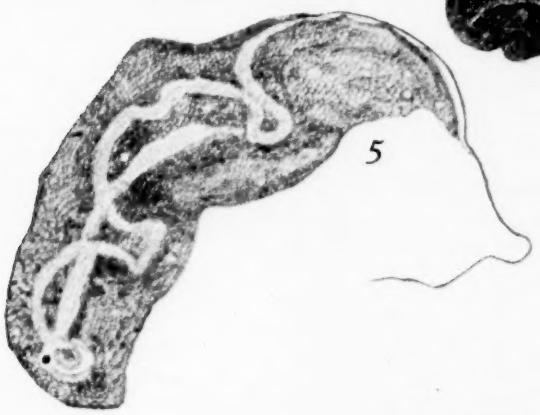
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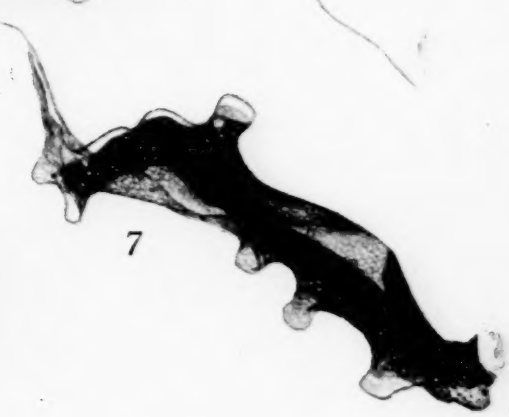
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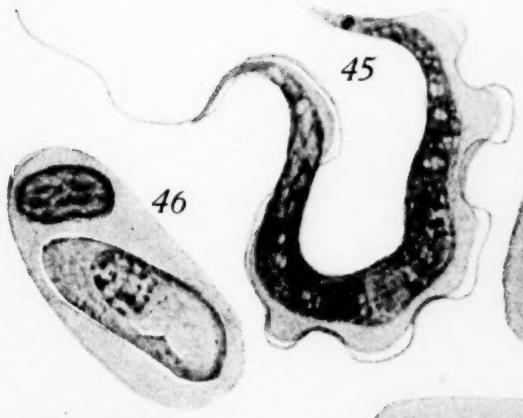
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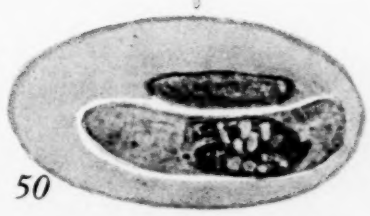
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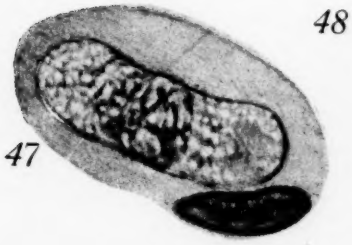
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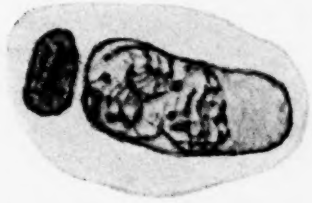
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PLATE XXV

PLATE XXVI

Fig. 9.—*T. loricatum.* Wide "hyla form," with folds unfolding. Nucleus indicated by a bent band.

Fig. 10.—*T. loricatum.* Short "hyla form." The nucleus is long and has chromatin massed at both ends. There is an unfolding of the edge of the body at intervals on one side of the parasite.

Fig. 11.—*T. loricatum.* Round form with short curved, narrow white band running from blepharoplast towards periphery. The nucleus has differentiated areas on both sides.

Fig. 12.—*T. loricatum.* Round form, dividing form. Two nuclei, and two blepharoplasts are present.

Fig. 13.—*T. loricatum.* Round form as in fig. 11, but with no differentiated areas in nucleus.

Fig. 14.—*T. loricatum.* Rounded form, about to divide, the flagellum having been lost.

Fig. 15.—*T. loricatum.* Dividing round form; two nuclei, two blepharoplasts, but the cytoplasm not quite divided.

Fig. 16.—*T. loricatum.* Small round division form.

Fig. 17.—*T. loricatum.* A still smaller form than the above.

Fig. 18.—*T. loricatum.* The parasite has divided into a group of 16.

Fig. 19.—*T. loricatum.* Rounded form with division of nuclei and blepharoplasts into four, but with no division of the cytoplasm.

Fig. 20.—*T. loricatum.* Small divisional form that has just acquired a flagellum.

Fig. 21.—*T. loricatum.* Herpetomonas-like form. Note the position of blepharoplast, nucleus, posterior granule, and flagellum.

Fig. 22.—*T. loricatum.* Trichomonas-like form. The anterior extremity is enlarged and round and from it go four flagella.

Fig. 23.—*T. loricatum.* Herpetomonas-like form with large anterior end.

Fig. 24.—*T. loricatum.* This is perhaps a variety of *T. inopinatum.*

Fig. 25.—*T. loricatum.* This is a common *inopinatum*-like form.

Fig. 26.—*T. loricatum.* An *inopinatum*-like form, wide at the level of the nucleus.

Fig. 27.—*T. loricatum.* A *sanguinis*-like form. Note the wide membrane, the position of the nucleus and the blepharoplast, and the shape of the posterior extremity.

Fig. 28.—*T. loricatum.* A "leaf-like form" (page 322). Note the position of nucleus and blepharoplast, and the shape of posterior and anterior portion of the body.

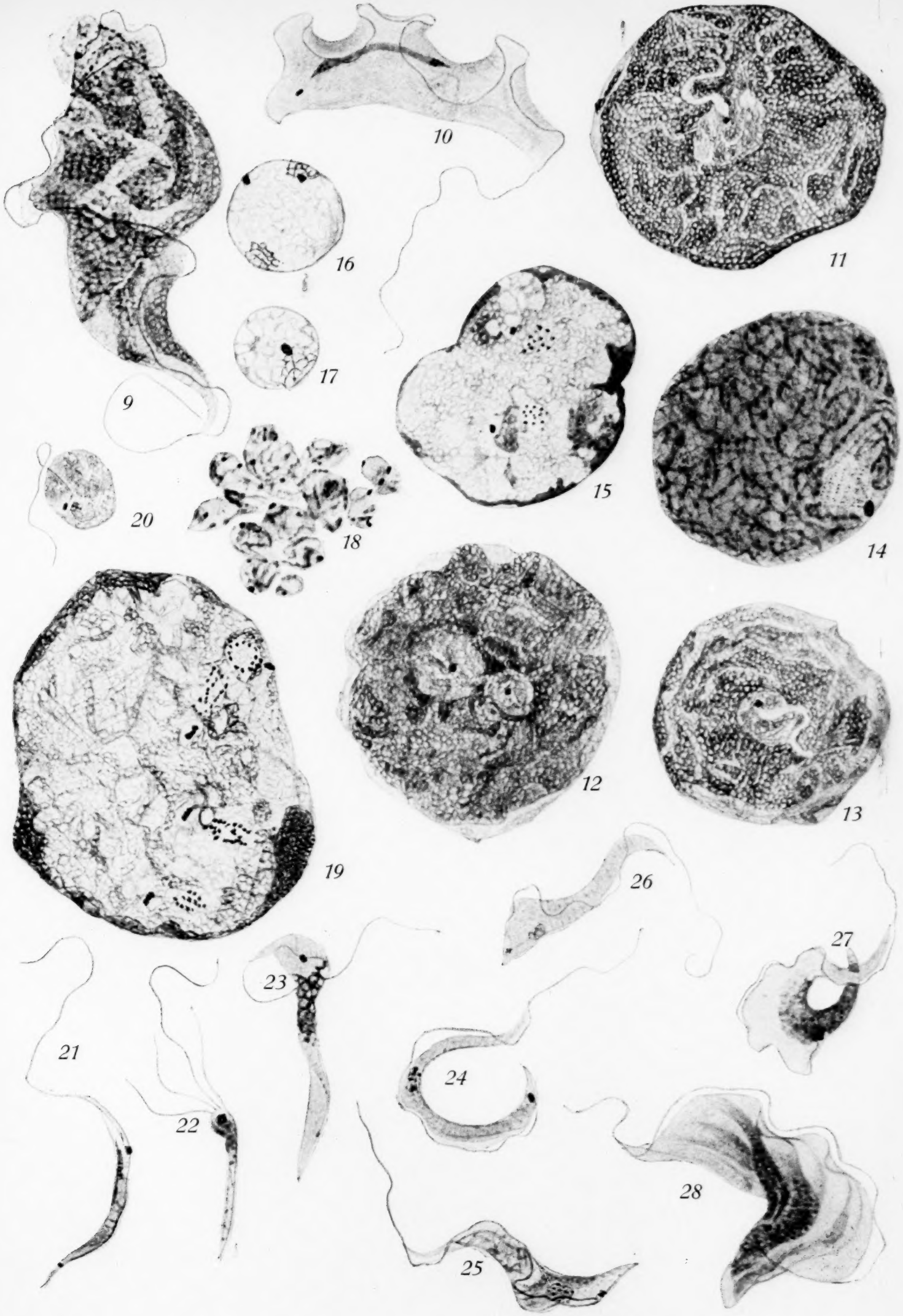


PLATE XXVI

PLATE XXVII

Fig. 29.—*T. loricatum.* "Leaf-like form" (page 322), with the posterior end arranged like a bud about to open. Note position of nucleus and blepharoplast.

Fig. 30.—*T. loricatum.* "Leaf-like form" unfolding. Note position of nucleus and blepharoplast, and shape of anterior and posterior portions of body.

Fig. 31.—*T. loricatum.* The "leaf-like form" has completely unfolded into an adult type of *T. loricatum.*

Fig. 32.—Intermediate form between the "leaf-like form" of *T. loricatum* and *T. mega.*

Fig. 33.—*T. mega.* A form with red staining granules in the cytoplasm. The nucleus is faintly stained.

Fig. 34.—*T. mega.* Same form as fig. 33, but with more red granules in the cytoplasm.

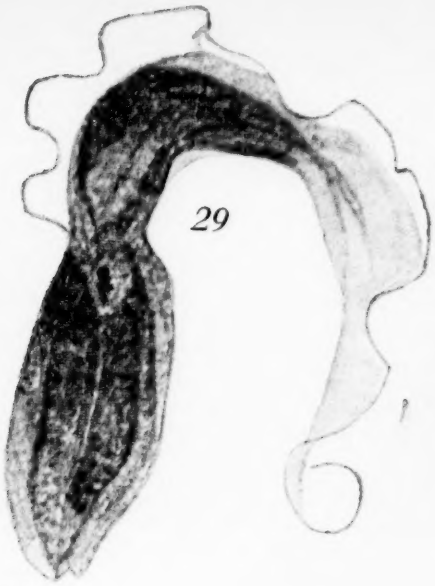
Fig. 35.—*T. mega.* The posterior part of the body is irregularly contracted. Note the shape of the nucleus.

Fig. 36.—*T. mega.* A somewhat generally contracted form. Note the shape of the nucleus.

Fig. 37.—*T. mega.* A very much generally contracted form. Note the shape of the nucleus.

Fig. 38.—*T. mega.* A variety of *T. mega* with coarse reticular structure.

Fig. 39.—*T. mega.* A much elongated form. Note the character of the nucleus, and the difference between the part of the body anterior to the nucleus and that posterior to the nucleus.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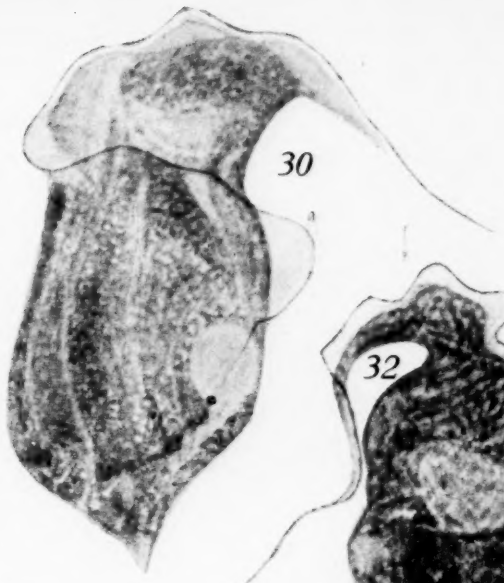
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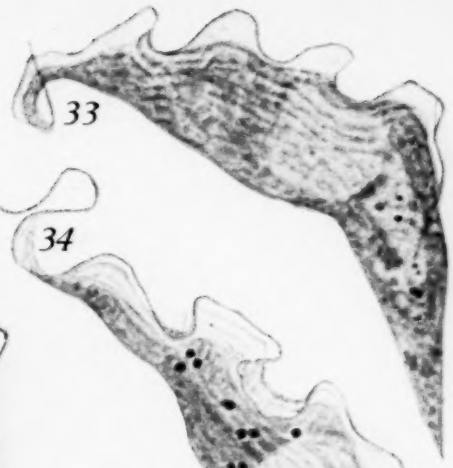
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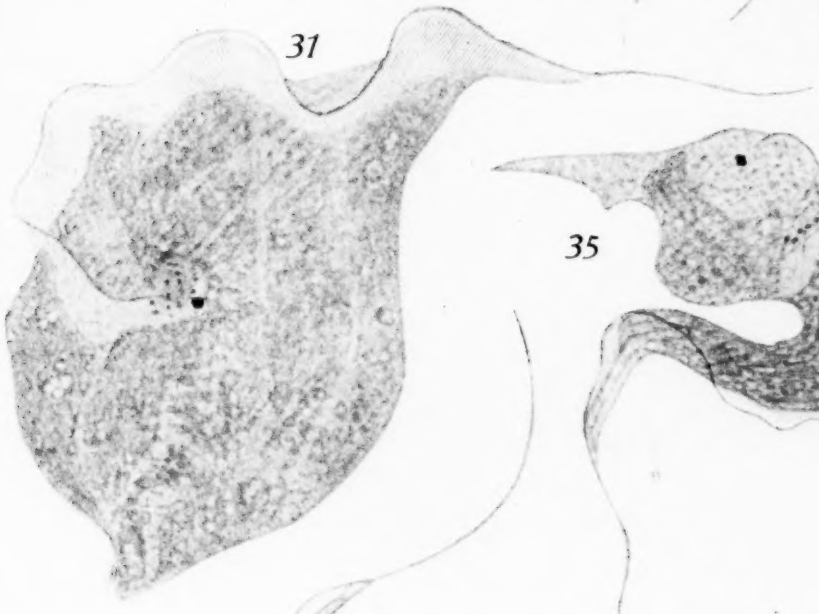
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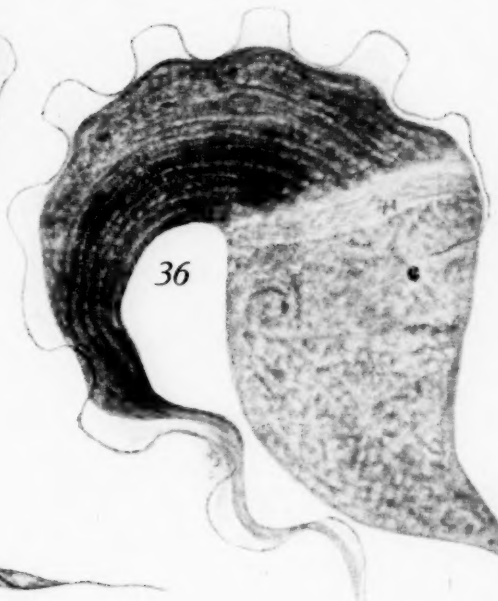
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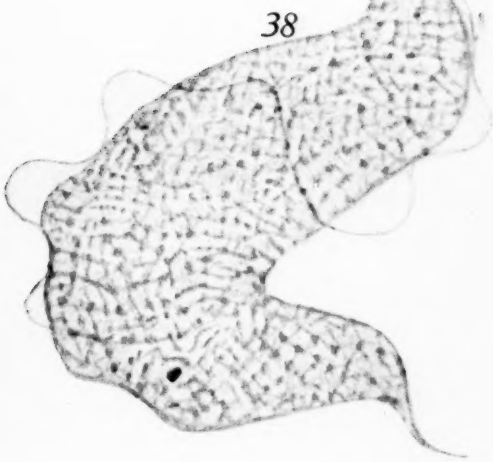
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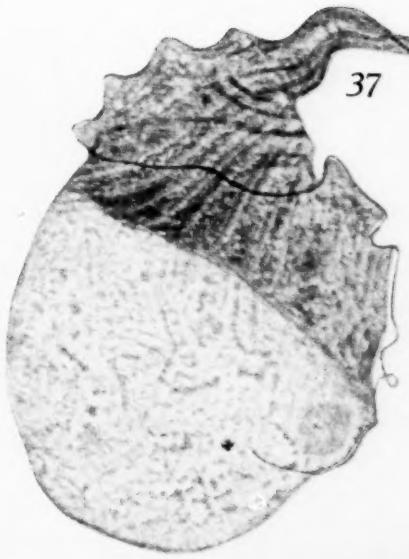
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PLATE XXVIII

Fig. 40.—Small form of *T. karyozeukton*, showing blue spiral line running from blepharoplast to posterior extremity, and the thick wide undulating membrane.

Fig. 41.—Medium form of *T. karyozeukton*, showing granule at posterior end of thickened edge of undulating membrane.

Fig. 42.—Large form of *T. karyozeukton*.

Fig. 43.—*T. karyozeukton* coiled, with the anterior end free.

Fig. 44.—Tighter coil of *T. karyozeukton*.

Fig. 56.—A curious appearance in a snake's blood (page 304) in a cell with a drepanidium.

Fig. 57.—The same lying upon a degenerated drepanidium.

Fig. 58.—The same with short rods of unequal length.

Fig. 59.—Intracellular drepanidium, showing excretion at both extremities within capsule.

Fig. 60.—Encysted drepanidium, found free in serum, showing excretion at the extremities.

Fig. 61.—Division form of drepanidium. The cytoplasm has divided synchronously with the chromatin.

Fig. 62.—Division form of drepanidium, dividing into two.

Fig. 63.—Division form of drepanidium, with the divisions almost separated.

Fig. 64.—Division form of drepanidium, five divisions.

Fig. 65.—Division form of drepanidium with four divisions.

Fig. 66.—Division form of drepanidium on the way out of the erythrocyte.

Fig. 67.—Common form of drepanidium, almost out of an erythrocyte, showing the eaten-out character of the host cell.

Fig. 68.—Common form of drepanidium, showing division of nucleus into two, connected by a red line.

Fig. 69.—Common form of drepanidium with dividing nucleus.

Fig. 70.—Common form of drepanidium with constriction at one end of the body, showing one method of movement.

Fig. 71.—Common form of drepanidium with two constrictions.

Fig. 72.—Young parasites of the small form of drepanidium.

Fig. 73.—Small form of drepanidium, showing nucleus and the blue spiral line running from a chromatin granule of nucleus to anterior extremity of body, × 4,000 to show structure in detail.

Fig. 74.—Small form of drepanidium, showing chromatin granules at posterior end, also a few anterior to nucleus, × 2,000.

Fig. 75.—Free division form of drepanidium.

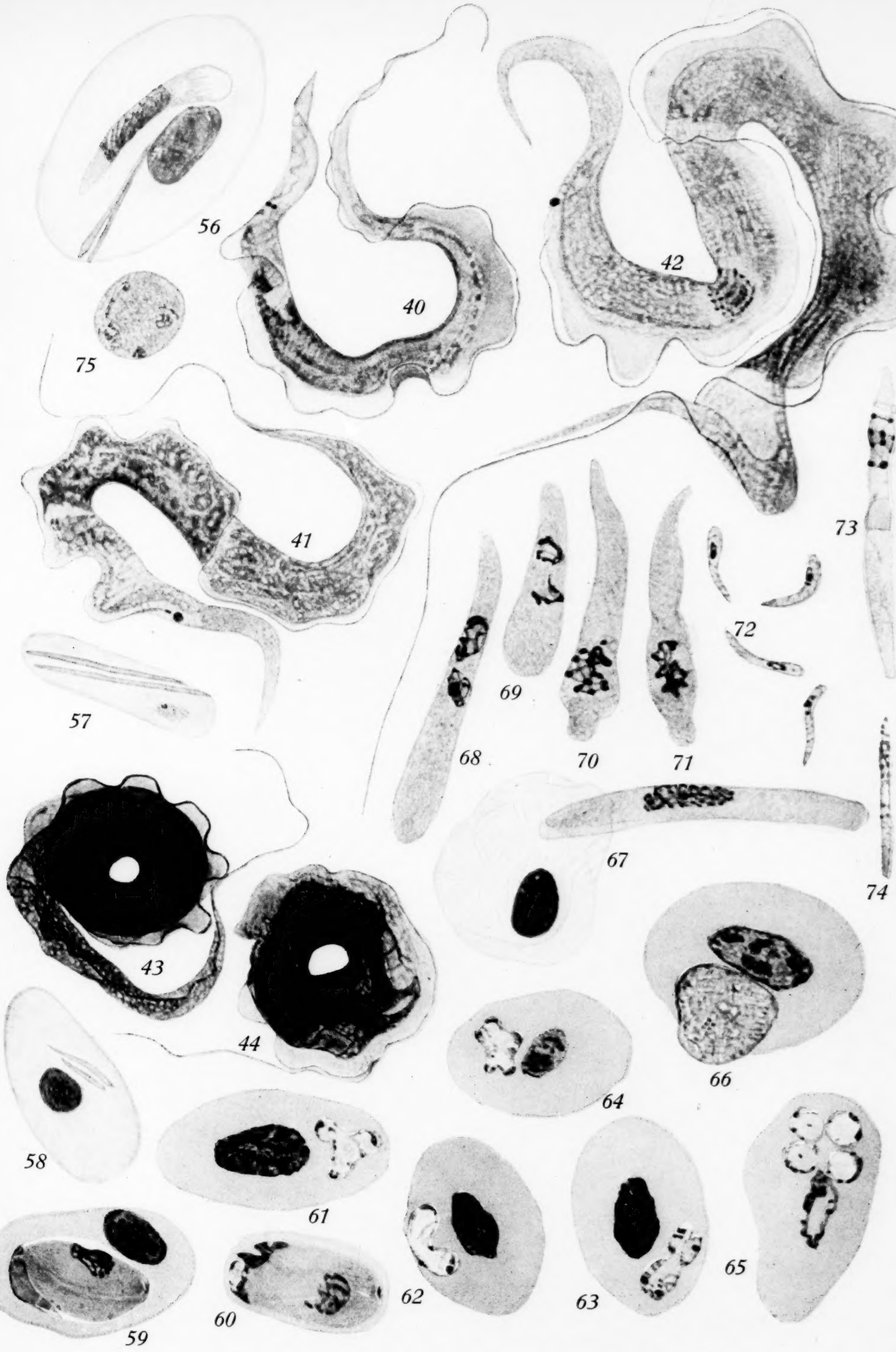

PLATE XXVIII

Plate XXIX

Fig. 76.—Division form with one larger mass of chromatin and several chromatin dots around it.
Fig. 77.—Division form with four masses of chromatin.
Fig. 78.—Division form with sixteen masses of chromatin.
Fig. 79.—Division form with twelve masses of chromatin and the division into well-defined "spores."
Fig. 80.—Double infection with rosette division forms.
Fig. 81.—Common form of drepanidium with division form beside it in same host-cell.
Fig. 82.—Common form of drepanidium with division form at an earlier stage of development.
Fig. 83.—Young form of large drepanidium.
Fig. 84.—Drepanidium showing one globule situated near the nucleus.
Fig. 85.—Drepanidium with about 100 refractile globules in it.
Fig. 86.—Large form of drepanidium.
Fig. 87.—Large form of drepanidium, showing body folded upon itself.
Fig. 88.—Large form of drepanidium, only partly folded upon itself.
Fig. 89.—Large form of drepanidium with 34 refractile globules.
Fig. 90.—Large form of drepanidium with two globules situated near nucleus.
Fig. 91.—Drepanidium with 11 globules situated near the nucleus.
Fig. 92.—Drepanidium with large refractile globules.
Fig. 93.—An unidentified parasite of frog with crystal.
Fig. 94.—An unidentified parasite of frog, showing a blue area sharply defined.
Fig. 95.—An unidentified parasite of frog, with crystal in nucleus.
Fig. 96.— „ „ with well-defined blue area.
Fig. 97.— „ „ apparently free in the serum.
Fig. 98.— „ „ with three crystals.
Fig. 99.— „ „ triple infection.
Fig. 100.—Cytomoeba free in serum.
Fig. 101.—Cytomoeba in cell.
Fig. 102.— „ „
Fig. 103.— „ „
Fig. 104.— „ „
Fig. 105.— „ „
Fig. 106.— „ „ round form.
Fig. 107.— „ „
Fig. 108.— „ „ round and long forms.
Fig. 109.— „ „ long form.
Fig. 110.— „ „ round forms breaking up nucleus.
Fig. 111.— „ „ round form breaking up nucleus.
Fig. 112.— „ „ long form.
Fig. 113.— „ „ long form.
Fig. 114.— „ „ round form.
Fig. 115.— „ „ long form.


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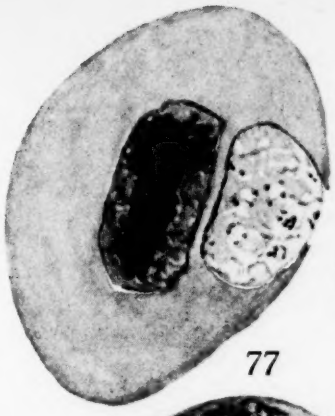
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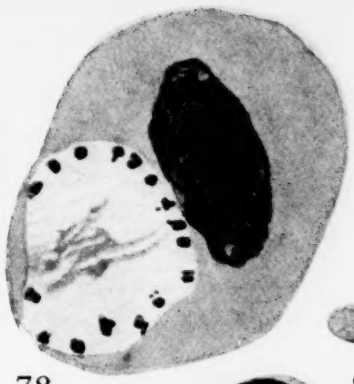
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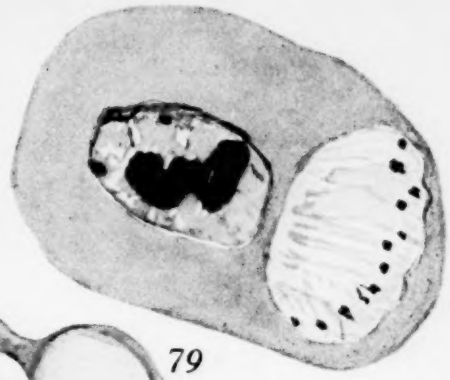
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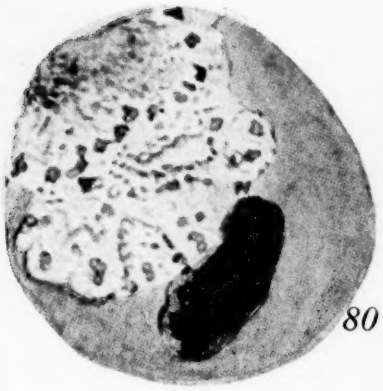
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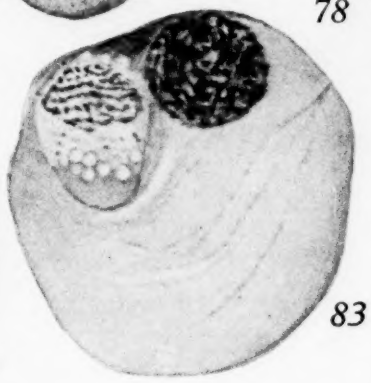
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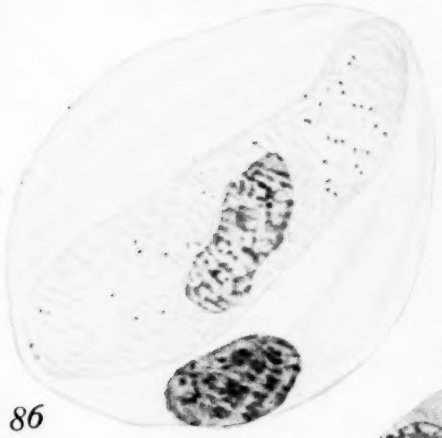
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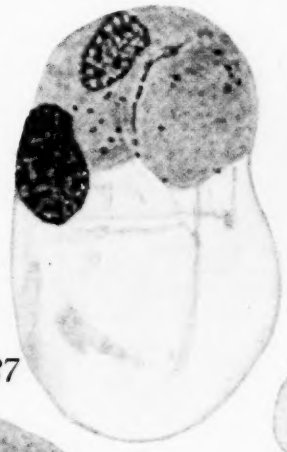
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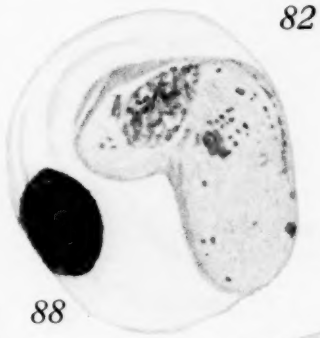
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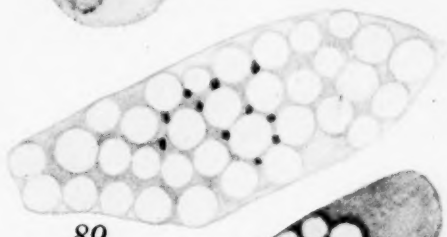
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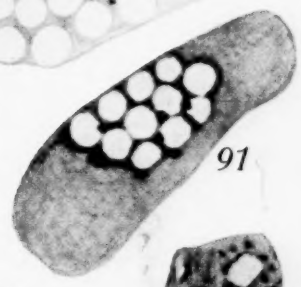
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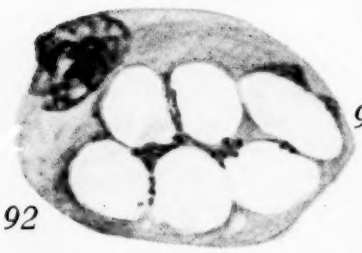
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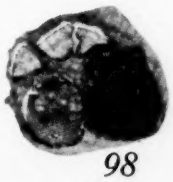
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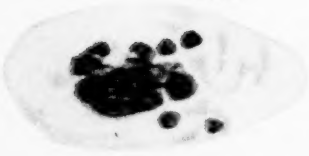
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PLATE XXIX.

Plate XXX

Fig. 1.—Small form, showing arrangement of the chromatin granules of the nucleus and of the granules of the blepharoplast.

Fig. 2.—Small form, showing early stage of division. The chromatin granules of the nucleus have gathered into two masses, and the granules of the blepharoplast have changed their position.

Fig. 3.—Medium form, showing arrangement of the chromatin granules of the nucleus and of the granules of the blepharoplast.

Fig. 4.—Medium form, showing striations.

Fig. 5.—Long form, showing arrangement of the chromatin granules and karyosome of the nucleus, the longitudinal striations, the granules of the blepharoplast and the vacuoles anterior to it.

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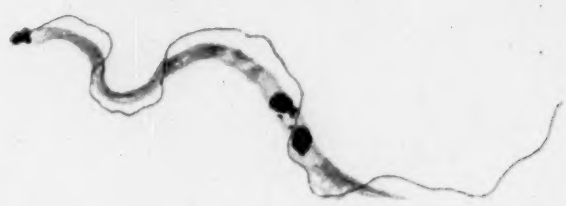

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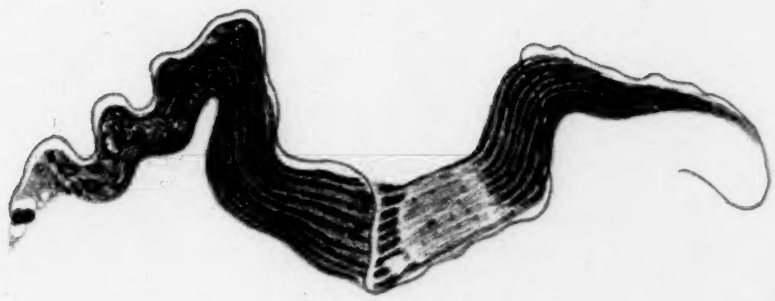

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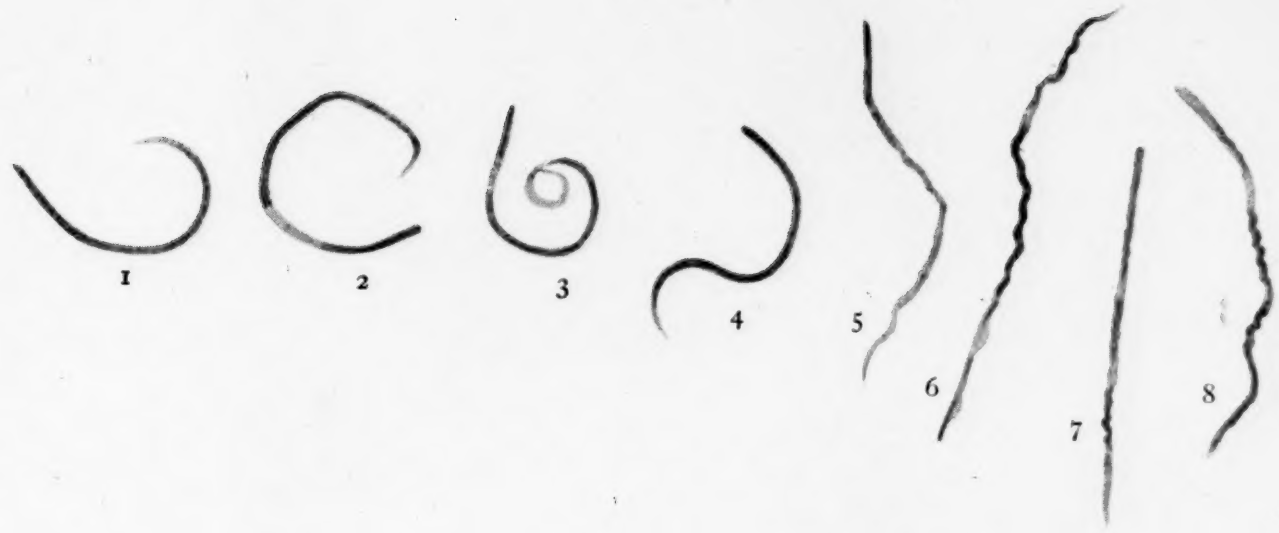
Dutton, Todd, Tobey.

PLATE XXX

Hemoflagellate.

PLATE XXXI

Spirochaeta jonesii from *Clarias angolensis*, see page 338.



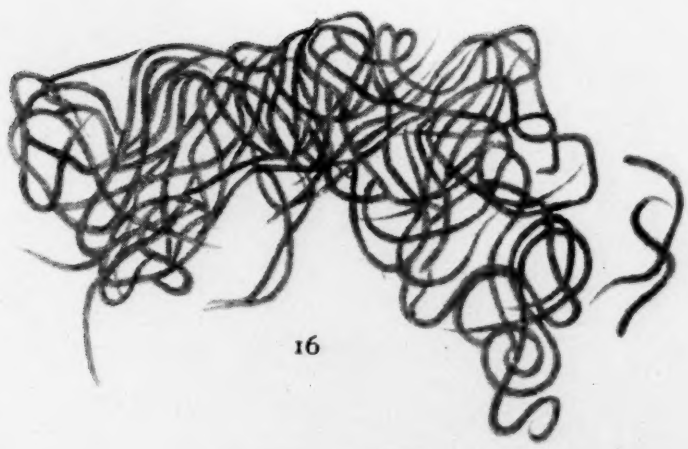


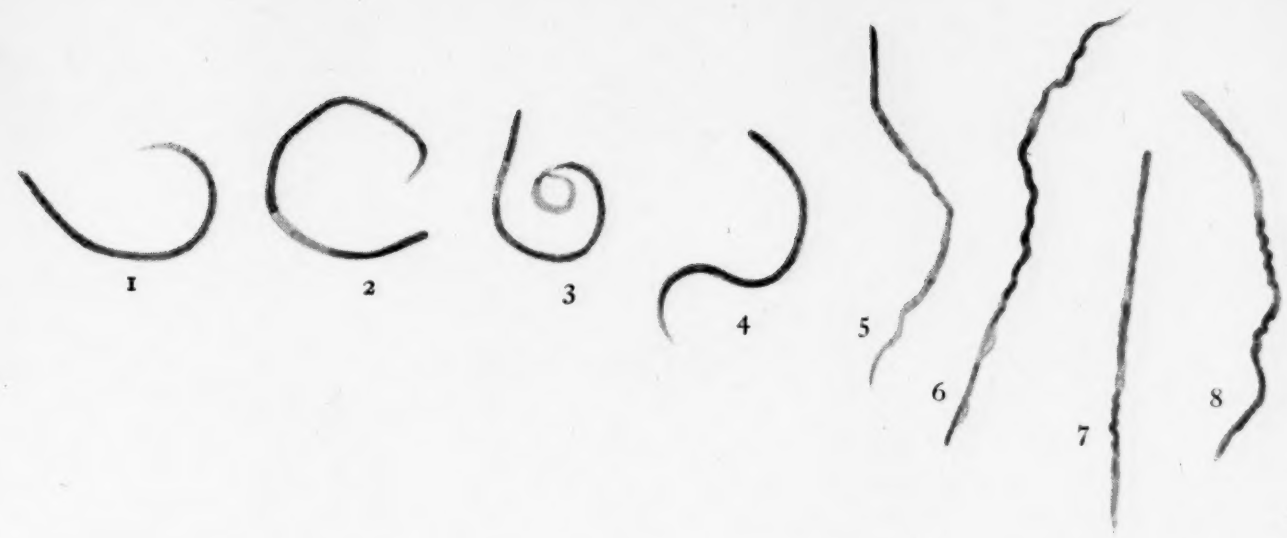
PLATE XXXI

Dutton, Todd, Tobey

Hemoflagellate

PLATE XXXI

Spirochaeta jonesii from *Clarias angolensis*, see page 338.

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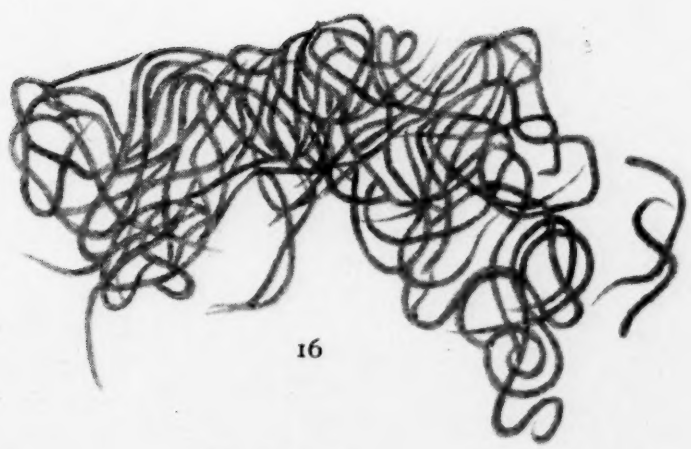
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PLATE XXXI

Dutton, Todd, Tobey.

Hemoflagellate.

PLATE XXXII

Myxosporidium heibergi from *Glossina palpalis*, see page 342.

Coccidium ——? from *Pyretophorus costalis*, see page 339.

MYXOSPORIDIUM HEIBERGI.

FIG. 14

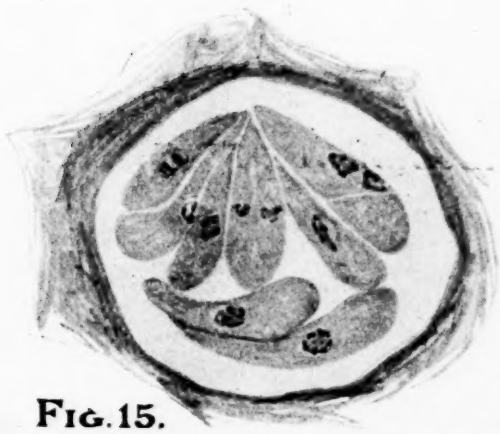


FIG. 15.

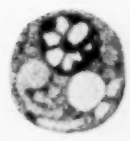
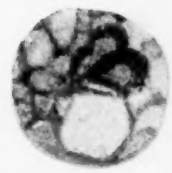


FIG. 16.



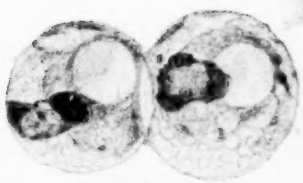


FIG. 17.

FIG. 18

FIG. 19.

COCCIDIUM OF PYRETOPHORUS COSTALIS.

YAWS

BY

C. W. BRANCH, M.B., C.M. (EDIN.),

MEDICAL OFFICER, COLONY HOSPITAL, ST. VINCENT, W.I.

(*Received May 13th, 1907*)

THE PREVALENCE OF SYPHILIS IN THE WEST INDIES

The most striking feature of the medical practice in the West Indies is the prevalence of tertiary syphilis and infantile inherited syphilis among the labouring class. In some of the islands this prevalence is quite extraordinary.

On the contrary, in the classification of diseases returned by the Colonial medical officers, as well as in the reports of hospitals, syphilis is credited with so few cases that it is incredible that the figures can be correct. In part this is due to the custom of classing diseases by the local manifestations, e.g. iritis, necrosis, paralysis, &c. The cases are thus scattered through the classification, and even if their true nature has been recognized it is not shown. But it is nevertheless a fact that great misconception does exist in the minds of many of the medical profession in the West Indies, and no doubt elsewhere in the tropics, as to the extent to which syphilis is responsible for the sicknesses that occur. The chief causes of this failure to recognize the disease are ignorance of the original nature of syphilis and the tendency to regard it as a venereal disease.

Failing to attribute a given lesion to syphilis the medical man naturally turns to tubercle, the effects of which on the natives of the tropics are in consequence vastly over-rated. Tubercular bone and joint disease is rare among negroes. Lupus I have never seen, though I have seen cases classed as such, the scars and subsequent history of which abundantly proved the syphilitic origin of the lupoid ulceration. Phthisis is very common among the negroes in some West Indian Colonies, but in view of the undoubted rarity of other

tubercular troubles, I am inclined to believe that a great deal of the phthisis is really syphilitic. I have several times been agreeably surprised by the recovery of a phthisis case. Without actually diagnosing syphilis, one gets such a belief in iodide, and uses it for such a wide range of complaints, that some of the lung cases have had the good fortune to be accidentally cured in this way. It is a safe thing to treat all supposed phthisis with iodide if the tubercle bacillus cannot be found or if there is no opportunity to search for it. But even after discounting for pure syphilis of the lung, there remains a number of tubercular cases quite out of proportion to the amount of tubercle of other tissues.

With respect to the West Indies I can personally vouch for the prevalence of syphilis in the islands St. Croix (Danish West Indies), St. Kitts, Nevis, Antigua, St. Lucia and St. Vincent. In Barbados I think there is not so much of grave syphilis, but the disease is widely spread nevertheless. Dr. W. J. Branch, after 25 years' practice in St. Kitts, said in reply to a query, "nearly every black or "coloured person on St. Kitts has, or has had, syphilis in some shape "or other, congenital, acquired or both."[1] I had five years' experience in district and hospital work in that island, and can assure the reader that though this estimate is true, the condition of the population of St. Vincent is worse. Here I have seen a man in middle life with tertiary scars dating from childhood, a scar of an old penile chancre, and a new eruption of secondaries. That is to say, in St. Vincent people may be "thrice dipped" in syphilis. Second infections are the rule of life if an individual comes to mature years. The combination of secondaries and tertiaries is very commonly seen.

In my annual hospital report for 1902-03 I wrote:—

"When to this moral state is added a profound and universal "saturation with syphilis and depletion by ankylostoma, it may easily "be understood that the present labouring population of St. Vincent "is as diseased and pauperized as any in the world. The effects of "syphilis depend on the nutrition of the patient; so that where there "is a soil as suited for the exuberant manifestation of the disease as "obtains here, it is not surprising to note the disablement and "increasing pauperism due to syphilitic ulceration and necrosis, "parasyphilitic paralysis, and degenerative neurosis. . . . . .

1. Nicholls' Report, page 167.

"Medical men from abroad, visiting St. Vincent, are struck by "the prevalence of disfigured and noseless faces, and the pauper "asylum is a museum of remnants left by syphilitic disease and the "surgeon's knife."[1] This is a lurid picture, but it is paralleled by the condition of more than one other West Indian colony.

Dr. Blanc, of Tobago, bears witness to the extensive spread of syphilis in that island. "In connection with this question, I may say "that in Charlotteville and Speyside, where yaws was so prevalent, "a very large proportion of the population suffer from syphilis."[2]

Dr. S. Branch, in his hospital report from St. Lucia for 1904-05, speaks as strongly of the prevalence of syphilis there as I do of St. Vincent.

These, after all, are only statements of opinion, but though figures are proverbially deceptive, I am able to adduce some statistics in support of my assertions with respect to St. Vincent. We may take these as practically representative of the state of affairs in the West Indies generally; for with few exceptions they all belong to one type; the poverty, the race of the masses, the climate, and the geologic structure are the same.

It was agreed by the Medical Officers of St. Vincent that all cases of tubercle and syphilis were to be classed as such in the monthly returns of cases treated, and not scattered under the different organic systems as local diseases. This has been done since July, 1905, and I can show some reliable figures for twelve months' district work in the Colony. The population of St. Vincent is estimated at 45,000. The hospital has a daily average of 55 to 60 patients. In the four years I have been in charge of the hospital there have been 3,269 admissions for all causes. This number is rather swollen by the cases of ankylostomiasis, many of which are admitted twice or several times for two days in order to take thymol. In these four years there have been 630 admissions for syphilis, of which 42 were for primary. Only about 20 of these cases were suffering from yaws, so that I have not materially swelled the total by including this condition with syphilis. The syphilis cases therefore form about 19 per cent. of the total admissions. When it is remembered that one naturally avoids filling one's hospital beds with chronic cases, such as those

1. Hospital Report, St. Vincent. Colonial Reprints, No. 20.
2. Nicholls' Report, p. 166.

suffering from tertiary ulceration, and that ankylostomiasis has run the total to an abnormally high figure, it must be admitted that this percentage shows well the prevalence of syphilis.

For some time I conducted an out-patient department at the hospital, as a single-handed effort to deal with some of the tertiary cases of the island. Between July, 1903, and March, 1905, that is, in twenty-one months I treated 600 cases of syphilis.

In the four years of hospital work referred to, a large number of amputations have been necessitated by syphilis. Of 51 amputations of the leg at the seat of election, 27 were performed for syphilis and 11 for elephantiasis. And even of the latter several were brought to the knife by extensive syphilitic ulceration.

In the reports of the Medical Officers for the district work for twelve months—July, 1905, to June, 1906—no less than 1,996 cases of syphilis are returned. This for a population of 45,000 is equal to 4·4 per cent. per annum of the inhabitants. Of these I attended myself 539 cases, which form about 21 per cent. of all the district cases treated by me in that time. It must be understood that these figures referring to syphilis have not been swelled by the inclusion of the cases of yaws. Though it is true that only one Medical Officer in St. Vincent now returns yaws as such, yet this condition is not often brought to our notice, and the few cases of frambesial eruption which we have treated would not affect the general truth of the figures. The one Medical Officer referred to whose district is that most infected with yaws, only returned 24 cases in the twelve months.

There is no compulsory medical certification of death in St. Vincent, except for infants under one year, but nearly all the deaths are certified by medical men. I went through the register for two years—March, 1904, to March, 1906—to pick out and classify the causes of death. From the vital statistics I may quote the following:—

Total deaths	1,418
Still Births	233
Syphilis	56
Hereditary Syphilis (infants)	149

Syphilis, therefore, causes on the average about 14 per cent. of the deaths in St. Vincent.

A high infant mortality (25 per cent. of the total deaths in St. Vincent) and a large number of still-births are observed all over the West Indies. These alone are evidence of the prevalence of syphilis, though there are other causes—illegitimacy, ignorance and poverty—which contribute to raise these rates.

Hereditary syphilis in infants is a matter which presses hardly on Government Medical Officers who are required to attend the children of labourers free, as they do in many West Indian Islands. In the twelve months' period referred to above, I saw 110 infants suffering from inherited disease out of a population of about 5,000. In the whole Colony in the same period there were returned 512 cases of hereditary syphilis. This is in a higher ratio to the total cases of syphilis than in my practice. I attribute this difference to my own tendency to class syphilis of children and young persons as acquired, only counting syphilis in infants as certainly or probably inherited. Comparing the ratios otherwise, I find a striking agreement in the figures relating to syphilis returned by myself and the other Medical Officers.

To recapitulate the proofs of the prevalence of syphilis in St. Vincent, we note

1. 19 per cent. of the admissions to hospital are for syphilis.
2. 4·4 per cent. of the inhabitants are attended yearly for syphilis by the district Medical Officers.
3. In a small hospital in a small colony no less than 27 legs were removed on account of syphilis in four years.
4. The cases of syphilis form 21 per cent. of the district cases seen by one Medical Officer.
5. Syphilis is responsible for 14 per cent. of the deaths in the Colony.

I take St. Vincent as a representative Colony in which the disease is well developed, though probably not much more so than in some other West Indian Colonies.

If now we examine the stages of syphilis seen by the medical men in St. Vincent, we shall find a preponderance of the tertiary and what seems to be an inadequate proportion of the primary stage. The figures for the same twelve months' period are as follows:—

Primary	35
Secondary	373
Tertiary	1,076
Hereditary	512

I may admit that the primaries include several (say four) non-venereal cases returned by myself.

It is true that the primary lesion, even when venereal, is frequently a very trivial-looking thing to the patient, but many such sores are aggravated by the dirty habits and incontinence of the black patients, and must come to hospital for surgical treatment. In St. Kitts we operated very often on such cases. There are, therefore, probably not many chancres of the genitals, in St. Vincent at any rate, which are not brought to the surgeon's notice.

This paucity of venereal chancres in the tropics is referred to by Numa Rat, whose experience extended to the West Coast of Africa as well as the West Indies. "And I may add that it is very rarely "indeed that I have seen the initial lesion of syphilis in a black man "in any country. Either the syphilitic chancre in the negro is a much "less serious affair than it is in a European, or the former considers "it too trifling, whatever its severity, to trouble the doctor about. It "is reasonable to assume that in a place like St. Kitts, in which "syphilis prevails so generally, the initial lesion of the affection would "be modified by the presence of an inherited syphilitic taint."[1]

It may justly be contended that 31 venereal primaries in 1,996 cases of syphilis is an inadequate proportion. And, again, in four years only 42 cases of primary have been admitted to hospital. It seems certain that the venereal chancres do not account for the amount of syphilis in the Colony, and it will be my endeavour to suggest an explanation in the following pages, which I believe applies equally to all tropical countries.

TERTIARY SYPHILIS IN EARLY LIFE

While we were speaking of tertiary syphilis, the reader no doubt has had in his mind the syphilis of adults. But we have now to consider another phase of the question. A great deal, if not most, of the tertiary in the West Indies is seen or has begun early in the life of the patients, and much of the juvenile syphilis is of a very severe character.

The large infant mortality indicates what has become of most of

1. Report on Anguilla. Colonial Reprints, No. 19.

the inherited syphilis, and it is well known that nowhere is the occurrence of inherited taint in proportion to the number of infected parents. If this were not so the human race would have disappeared at an early period of its existence. I have rarely seen a black or coloured child over three years of age whose syphilis was undoubtedly inherited. Eye lesions, sabre shins and cranial malformations occur, but by no means commonly among the juvenile cases of syphilis. Hutchinson's teeth I have never seen in a dark-skinned person, and doubt if this mark affects the negro race.

Moreover, the manifestations of disease in young persons are usually of a severity that one does not associate with inherited taint. Under the unfavourable conditions of child life among the black peasants, the bad cases of inherited disease die early. The milder cases we should not expect to run to such virulent tertiaries as we commonly see. Those writers who try to prove that some of these lesions, such as necrosis of the palate, are not caused by syphilis urge the youth of many of the patients, and thus they indirectly admit the improbability of their being due to inherited taint. I can therefore produce these writers in evidence. J. Numa Rat is perhaps the most careful and accurate observer of yaws. He does not believe that this condition has any relation to syphilis, but finding a prevalence of lesions apparently identical with those of tertiary syphilis, he did not attribute them to inheritance, but to yaws itself.

The very common lupoid ulceration is rarely ascribed to syphilis in the West Indies for the same reason of the youth of the patients; it is called lupus. Yet it invariably gets better under iodide, and often spontaneously. Just as common in St. Vincent is ulceration and necrosis in the throat and nose, affecting young people more frequently than adults. In a recent correspondence in the Journal of Tropical Medicine, James Leys[1] and Numa Rat[2] discuss this condition, which the former found as prevalent at Guam in the Philippines as it is in the West Indies. It is said to be very common also in Fiji. Leys suggests the name "rhinopharyngitis mutilans" for the condition, and both the above writers agree that it cannot be syphilis because the patients are often young, fourteen to nineteen years. As a matter of fact, sexual life begins so early with the dark races that

1. Jour. Trop. Med. February 15th, 1906.
2. Jour. Trop. Med. May 1st, 1906.

a patient of sixteen years could easily have had time to reach this stage of tertiary from a venereal infection. And in my own experience in St. Vincent most of the few females I have seen with venereal primary were about or under this age. But I have seen rhinopharyngitis in patients as young as nine or ten years. Both these writers ignore the possibility of non-venereal infection in childhood, and neither even raise the question of inheritance.

The rhinopharyngitis as described by Leys and accepted by Rat is identical with that we have in St. Vincent. It is such a characteristic tertiary that it would be diagnosed as such without a moment's hesitation if seen in a single individual. But it is very common in some places, e.g. certain parts of the West Indies, Guam, Fiji, while in other places where there is just as much syphilis it is only occasionally seen. It is this peculiarity of distribution which makes the difficulty. I have pointed out that the damp, hot, wooded ravines of volcanic islands in the tropics are infested alike by yaws and rhinopharyngitis. The same climatic conditions determine the occurrence or favour the development of both. The point to which attention has not been sufficiently directed is that syphilis is modified by circumstances of climate, race, personal habits and constitution, &c., so as to manifest itself in various forms. In St. Vincent, for example, lupoid destruction of the face is the rule on the Windward side of the island, and necrosis of the palate and turbinated bones on the Leeward. It is incorrect to judge of syphilis of dark races in a tropical hot-house by the standard of syphilis in Europe. So certain is it that rhinopharyngitis mutilans is a tertiary that its presence in Fiji should be conclusive that the supposed freedom of that group from syphilis was a delusion, if such proof was ever seriously required.

The condition is this ; there is ulceration of tonsils, fauces, pharynx, palate or nose, progressing to destruction and accompanied usually by necrosis of the hard palate, the turbinated bones and vomer. Any or all of these parts may be involved. The nose at length may fall in, but the ulceration does not usually reach the skin of the face. One may see the buccal, nasal, and pharyngeal cavities thrown into one space, lying between the tongue and the base of the skull, and lined by a vast green-gray ulcer relieved by blackened remnants of necrosed bone. The condition does not appear to kill readily, for the

1. Jour. Trop. Med. May 15th, 1906.

patients continue slowly losing tissue for years when untreated, and it may heal even after most extensive destruction has taken place. There is just as frequent syphilitic ulceration, but of the skin, in other places where the rhinopharyngitis is not so common.

Hutchinson describes the same lesion as being not infrequent in the young, and attributes it to inherited taint. It is remarkable, however, that he does not usually find the other signs of heredity, notched teeth and keratitis, in such cases. In view of the facts which I shall later on present, I have taken the liberty of disagreeing with the master's opinion, and believe that this severe tertiary is usually the result of acquired syphilis.

According to Hutchinson, the tertiaries of inherited syphilis commonly seen are interstitial keratitis at about the age of puberty; deafness; periosteal nodes between the ages of, say, eight to ten years, sometimes ending in necrosis; lupoid ulceration and ulceration of the pharynx and palate. I have only seen three instances of deafness in juvenile syphilitics in St. Vincent. Interstitial keratitis is rare; I can only remember one case. Perhaps the negro race is not prone to it, though iritis and other eye affections are sometimes seen in acquired syphilis of blacks. Periostitis and synovitis in young persons and children are common enough, but these belong as much to the acquired disease. Lupoid ulcerations and ulceration of the nose and throat are, as we said before, very common in young persons. These also occur in acquired syphilis.

But it is not, I believe, recognised that cutaneous gumma and subcutaneous gumma leading to gummatous ulcers occur or are at all common in the inherited disease. So frequent is the former in the West Indies that it is well known to the natives under the names "blind-boil" and "blue-blister." It is just as common in children as in adults.

The terrible and long-lasting ulcers often met with in the young peasants are sometimes superficial and lupoid or serpiginous, but more commonly they are deep subcutaneous gummata. One may see the early stage when there is a fluctuating tumour so likely to be mistaken for abscess. I have myself cut into such a gumma in a child of eight years, under the impression that it was a cold abscess, and found the characteristic soft pale tissue exuding a gummy secretion from the cut surface. The frequency of these tertiaries is, I think,

conclusive of the occurrence of youthful acquired syphilis, for if even possibly hereditary, they can only be rarely so.

On the whole, therefore, while there is a great deal of congenital syphilis of infants, yet I am not satisfied that the amount of youthful tertiary which we see can be accounted for by inheritance. As before remarked, most of the congenital disease disappears by the death of the infants. What is left is presumably of a milder type, and would not produce such virulent and lasting tertiaries. When it can be shown, though this is anticipating my argument, that innocent syphilis is of frequent occurrence (apart from yaws), I feel I am justified in saying that the juvenile tertiary as seen is not the result of inherited taint.

I have for some time been in the habit of jotting down short notes of syphilitic cases, though this has not been kept up regularly, and there are long and frequent hiatuses in my record. Yet out of the notes, such as they are, I can place before my readers enough cases to illustrate the several points to which I wish to call attention.

I think it will be agreed that the cases following here read much more like examples of acquired than hereditary syphilis in spite of the youthfulness of the patients. I have been limited by selecting cases in which there was no history of yaws.

CASE I.—B. I.; 20 years; Black. No note of earlier history.

June 6th, 1903. Pigmented scars on both legs. Deep adherent scar over the lower end of the left fibula. Has had an ulcer of the right leg for some years. At present this is an enormous sloughing ulcer involving half the circumference of the leg, which is much enlarged. The rest of the circumference of the leg is cicatrized. Refused amputation. This is clearly a case of tertiary of many years' standing in acquired disease. Yet at the time when she must have had the primary she was rather too young for venereal infection.

CASE 2.—W. S.; 13 years; Hindu.

June 1st, 1906. Deep scars on both knees. On the right ankle an extensive and adherent scar. Said he had this more than four years ago (dated by the volcanic eruption). A large foul tertiary ulcer covers the back of the right foot. This has been of two years' duration. The tertiary here dates from the age of nine years.

CASE 3.—D. S.; 10 years; Black.

July 10th, 1903. Pigmented scars of "blind-boils" on both legs. Several superficial ulcers on the left leg. Swelling of left ankle, and periostitis of lower end of left fibula. Enlarged glands in both groins, those of left side tender. Hard glands on both epicondyles of elbows.

The blind boils are small indolent ulcers about 6 to 10 mm. wide, and circular. They are commonly multiple and symmetrical in arrangement. They begin as a pimple which breaks down and suppurates, leaving a shallow punched

ulcer, which is very resistant to treatment. They seem to belong to the "intermediate" stage of Hutchinson. This case is one of early tertiary in acquired disease, at an age which precludes the possibility of venereal infection.

CASE 4.—A. M.; 12 years; Black. Denies yaws.

July 2nd, 1903. Left tibia sabre-shaped. Right with a very large node. Large scar on back of right thigh. Large ulcer inside the upper lip. Columna nasi has disappeared, and a large ulcer covers the surface of the nasal cavity, eroding the alae nasi. Several scars of previous skin gummata on the inner side of nates. Here it may be admitted there is a probability of heredity indicated by the sabre shin.

CASE 5.—W. C.; 18 years; Black. Has been five years in the Pauper Asylum as a hopelessly disabled person.

October 11th, 1902. Left arm has been amputated below the elbow. Elbow scarred and ulcerated. Left leg is a scar from hip to toe, with the knee and ankle contracted in web-like keloids. Right leg is scarred below the knee. Both feet are masses of ulcers and bone disease.

After more than two years' treatment with iodide, and amputation of the left thigh, he was completely healed and left the Asylum. He has remained well to the present. I think that hereditary syphilis is not known to produce so frightful a condition.

CASE 6.—J. L.; 22 years; Black. Sent to the Pauper Asylum at the age of 16 years.

October 17th, 1902. Both knees and elbows stiff and contracted; extensive scarring and lupoid ulceration on both shins. Multiple nodes on forearms and humeri which give them a distorted appearance. Hard palate perforated. Deaf.

This case had since healed, but lately developed intense headaches and very marked mental dulness. There is no question of venereal infection in one who is already hopelessly disabled at 16 years.

CASE 7.—J. B.; 22 years; Coloured. Was disfigured at the age of 14 years. Was sent to the Pauper Asylum at 17 years.

October 17th, 1902. Nose is gone, lower lids retracted by scarring. Side of upper lips drawn upwards. Face scarred generally. Left leg extensively scarred by gummatous ulcers on the lower half of the shin. Ulceration of throat. Since healed under potassium iodide and sent out. Here again the early age at which the patient was considered incurable precludes the venereal infection.

CASE 8.—W. S.; 9 years; Black.

October 23rd, 1902. Large keloid on right elbow and a scar on the forearm. Large keloid scar on left knee. Scar on outer malleolus. Large gummatous ulcer and scars on right ankle. The scarring here indicates deep or subcutaneous gummata which had been appearing during several years.

CASE 9.—R. S.; 14 years; Hindu.

November 7th, 1902. Extensive scar all round the left elbow and half of the arm. Extensive scar on the back of the hand, and a scar on the wrist. Two fingers contracted and a few small ulcerated spots on the fingers. Extensive scars at the back of the right elbow, back of lower forearm, and back of hand. Large scar over the front of left knee, and another of the calf. Scarred all over the lower end of the tibia. Foot a distorted mass of scars and bone disease, with one large and several small ulcers. Scars on right knee and on lower end of tibia. Large ulcer of ankle. Seven superficial scars on cheek, nose and lip. Septum nasi gone. Was treated in hospital with a view to amputation of the

left leg, but when considered fit he declined operation and was discharged. He has not been heard of since. This is a class of case that is never seen in Europe. I have notes of several as severe cases in St. Vincent.

CASE 10.—L. B.; 9 years; Black.
November 21st, 1902. Perforating ulcer at junction of hard and soft palate. A very young case of "rhinopharyngitis."

CASE 11.—C. A.; 16 years; Black. Says, "never had yaws."
March 1st, 1906. Scar of velum palati with a perforation and a cleft of the palate. Scar on the face, one on the arm, and several on the legs, of tertiary ulcers. Two of these involved loss of bone from the tibia. Gummatous ulcer of one foot and the other leg.

CASE 12.—A. S.; 12 years; Black.
February 11th, 1903. Fistula of lachrymal duct from disease of maxillary bone. Left ala nasi deformed and nostril contracted by a scar. Cartilage of septum has gone. There is an ulcer of soft palate and fauces. Uvula has disappeared. Another young case of "rhinopharyngitis."

CASE 13.—E. W.; 12 years; Black. Had disease of the nose two years ago.
November 22nd, 1902. Ulcer and necrosis of palate. Ulcers of right thigh, knee and skin. Ulcer and bone disease of first metatarsal. Ulcers of left thigh, knee and shin. Both knees contracted and legs wasted. Ulcers of right arm and hand. Extensive scars on left forearm; large periosteal node on fifth metacarpal. Some wasting of both arms.

This case is known to have had yaws when two years old. Cases of tertiary with history of previous yaws may be multiplied indefinitely.

Among 17 children under the age of 15 years suffering from tertiary syphilis, yaws was admitted in the case of 12. Of the others only two had marks of inherited taint. I regret that I cannot quote a greater number, but one has been so in the habit of expecting the history of syphilis, that enquiry was not often made on this point, and I can only find a reply to this question in the notes of seventeen children.

NON-VENEREAL SYPHILIS

How often do we hear it inferred that a condition cannot be syphilitic because the patient is young and there is no evidence of inheritance. And how often is there a reluctance to attribute syphilis to an adult, from a feeling that this is an unjustifiable slander of his character. Yet thoughtful writers have warned us against this attitude.

> "The physician must forget the local primary lesion, and "put aside the idea that the diagnosis of syphilis carries with "it any stigma of impurity." (Fagge, by Pye Smith.)

I cannot state the truth about the West Indian negro better than by quoting my own words from a report already referred to.

"This applies with far greater cogency in the West Indies, where "the presence of a primary lesion on the genitals of an unmarried "peasant girl implies no immorality. Among the people there is, in "a true sense, no sexual immorality. Coitus among children before "puberty is as common as kissing among European children. The "normal sexual life of the adult is one of transient concubinage, "which does not restrict casual intercourse any more than does the "occasional marriage. The mother of a large family of unknown or "various paternity finds it very tiresome in the daughter of sixteen "years to add another infant to her burden. But that is all. She "accepts the grandchild with simple faith, and tells you that God "sent it."

Four years ago I was asked by the police to see a case suspected to be suffering from smallpox (or Trinidad varioloid varicella). I found the patient, a young woman of decent coloured class, to be a case I had already been treating for syphilis within the last few months.

But the interesting feature was that two little girls, aged about seven and eight years respectively, in the house had now an eruption of papular vesicular syphilides. Not long ago I attended the daughter, aged nine years, of a coloured gentleman. She had been ailing and getting thin for some months, had had frequent and sometimes severe rheumatoid pains, and a persistent rash of papules. She had been under treatment by another physician without improvement. I offered no diagnosis, but prescribed a hundred powders of grey powder and saccharated carbonate of iron. The rash rapidly cleared up, and the general health became robust many weeks before I permitted the discontinuance of the powders. These were instances of non-venereal infection among clean, well-cared for children.

About the same time that the first case above was seen, I was in consultation on a black peasant girl of sixteen years, suffering from a profuse pustular rash, which was thought likely to give rise to a smallpox scare. My colleagues both diagnosed yaws. I said syphilis, and from my point of view we agreed. But one of the others, a man with thirty years' experience of the West Indies, objected—"how could she get syphilis." I have been treating that girl off and on to

the present time for profuse eruptions, iritis, numerous suppurating glands in the neck, bone aches, periostitis. In fact, she has had nearly every early syphilitic symptom possible except nerve syphilis. But she has never had either a venereal chancre or a frambesial eruption.

It is most important that the profession, especially in the tropics, should realise the importance and frequency of extra-genital syphilis. We all know that surgeons and nurses get finger syphilis occasionally. In the island of St. Kitts, with at no time more than eight medical men in practice, and usually only six, there were in twenty-seven years five instances of finger syphilis in surgeons. These are well-authenticated cases.

When we consider how readily syphilis is inoculated, and how the habits of the poor in the tropics afford opportunities for inoculation, we shall not wonder that extra-genital primary occurs. In the West Indies the labourers, especially children, go barefoot, and are, therefore, frequently receiving bruises and scratches on the feet and legs.

The negroes never realise the fact of infection or contagion. This forces itself on our attention by the trouble they give when quarantine measures are to be carried out. They are too indolent or careless to make any effort to avoid or prevent infection, even when they acknowledge that a disease is catching. A child with yaws huddles together with the other children just as naturally as the adults will sleep or cohabit with lepers. In Tortola, while I was there, the only female who could be called a professional prostitute was an ulcerated leper. The syphilitic, the yawsy, the leprous and the healthy mix in free and intimate contact without a thought. Leprosy, like elephantiasis, may be the subject of reproach, but these diseases are not a cause for avoidance of physical contact. Yaws is hardly even a disgrace, except in the eyes of schoolmasters and other coloured folk of a better class than the labourers.

Under such circumstances, it is hardly wonderful that yaws can be extremely prevalent in a native community; and it would be miraculous if non-venereal infection did not occur where syphilis is almost universal.

I think observers in other longitudes, who see the people, not in hospitals, but in their huts, will find my description of West Indian

peasant life not unfamiliar, and if a look-out is kept, I am sure others will verify the existence of non-venereal syphilis for themselves. In places where yaws, under its various names, is prevalent, it will be found that frambesial eruptions and non-venereal syphilis of ordinary type are running together. In the drier and less-wooded localities the latter will probably be found the more common form.

It must be remembered that in inherited syphilis secondaries do not occur after the second year, and are usually over in the first year of life. Tertiaries are the only lesions by which inherited taint can be manifested in children of, say, three years and upwards. Jonathan Hutchinson lays down :—

" If secondary symptoms of the kind described are to occur at all, " they will show themselves in infancy, and in the vast majority of " cases within the first three months of life. This is a very important " fact. If a syphilitic infant survive the first outbreak, in the course " of from six months to a year the symptoms common to this stage " (the rash, snuffles, mucous patches, &c.) will wholly disappear, and " there will follow a period of some years during which no active " symptoms will occur."[1]

When, therefore, undoubted secondary eruptions and swollen glands are seen in children over the age of two years, they are almost certainly due to acquired disease, and this certainly rapidly increases with the age. Such children should be examined for the primary, which in the bare-footed is usually on the foot, most commonly on the outer malleolus. The indurated pigmented scar may be seen some time after the sore has healed, but these sores are often of long duration, and may be still unhealed months after they were infected with syphilis. They are often already indolent ulcers before the infection. I have diagnosed such primaries before the general symptoms were manifest, and the later eruptions have justified my diagnosis. More usually, however, one passes the sore by until the patient is complaining of rheumatoid pains or fever, for sore feet in children are so common that we cannot treat them all as potentially syphilitic.

For several years I have been impressed with the frequency of these extra-genital chancres in children, followed by secondaries of

1. Syphilis, Ed. 1901, p. 75.

the ordinary type, and some of my colleagues have adopted or confirmed my opinion. Such cases are seen more commonly in towns where yaws is never prevalent. I have seen a few such develop frambesial eruptions after having been diagnosed and treated as syphilis; the frambesiae appearing along with the other syphilides.

Nearly everyone familiar with yaws recognises what the natives all know, that there is a primary in yaws. No doubt this is often a fungating ulcer rather like a frambesia, but it may be identical with the foot chancre of ordinary type. Chancres on the penis not infrequently fungate in the same way, as Hutchinson points out. The foot chancre, out of which yaws also sometimes originates, is, in my experience, a small sore about 5 to 10 mm. wide, round, with indurated base and periphery and raised edges. It may be on one of the toes, the inner malleolus, the tubercle of the fifth metatarsal or back of the foot, but the commonest site is the outer malleolus.

In the case of yaws, the natives in some instances do not recognise the primary, which may be inconspicuous, as is often the case in syphilis; and then a large group of frambesiae, especially a horse-shoe group, may be called the "mother-yaw." On the other hand, a primary may really become the "master-yaw" or largest frambesia, by fungating and having a satellite eruption of papillomata around it.

It is perhaps unnecessary to insist that there is aways a primary in yaws. Numa Rat has made this quite clear, and the universal opinion of the inhabitants of yaws districts themselves leaves no doubt on the subject. Notwithstanding, it is urged (e.g. Manson, Tropical Diseases, p. 530) as an argument against the syphilis theory that the primary is "wanting in yaws."

The identity of non-venereal syphilis of the easily-recognised type, such as I first referred to, and the whole group of symptoms called "yaws," is so obvious that the peasants themselves bear witness to it. They include under this name other skin eruptions, besides the frambesiae, which they have observed to be associated with the latter. These have popular names—tubbo, corn-yaws, crab-yaws, pian-gratelle and dartres. The peasants speak familiarly of "yaws-pains." They point to where a patient did "ketch a yaws," who has never developed frambesiae, but had rheumatoid pains and vesicular syphilides, and is afterwards seen with tertiary.

It is well to consider some of these other yaws eruptions, which are so well recognised by the natives and described by writers.

Tubbo is a papilloma, pathologically the same as the frambesia, though unlike it in appearance, which breaks through the thickened skin of the sole, and is modified by the pressure of the hard epidermis, in the same way as the small papules of early psoriasis are modified when they appear under the skin of the palm.

Corn yaws in English-speaking colonies covers all discrete papular, vesicular, and even pustulo-vesicular eruptions.

Crab yaws is simply palmar and plantar psoriasis. It is sometimes severe, and becomes a moist dermatitis. More usually it is seen some time after the yaws, and is identical with the tertiary plantar psoriasis. It is chronic, and very resistant to treatment.

Pian gratelle, as described by Numa Rat, Alford Nicholls and others, is obviously the small papula of syphilis-lichen. It appears at an early stage in a profuse crop usually, and, as in European syphilis, it may reappear as a late secondary, and its presence indicates a severe infection. This tendency to recurrence and this significance of the papular eruption is insisted on by writers on both syphilis and yaws (cf. Jamieson, Diseases of the Skin, p. 544, and Alford Nicholls, Report, p. 285).

Dartre (or as Nicholls calls it, the macula or squama) may be perhaps in some instances the macular rash, which is the earliest skin symptom of syphilis. The colour of it cannot be seen on a black skin, but when severe and on unwashed skin it may possibly result in an exfoliation of the corneous layer of the epidermis; but as described it is the syphilitic scaly psoriasis. This is the earliest eruption in yaws, and is very common. It is frequently coincident with the papular eruption—lichen—when that is present, and sometimes lasts to the stage of the frambesial eruption. Its appearance is absolutely characteristic of scaly psoriasis.

All these obviously syphilitic conditions are recognised by the natives to be essentially parts of yaws as a disease. A patient is said to have yaws on the strength of any of these, even by medical men, without the frambesial eruption.

Alford Nicholls and Numa Rat, who have made long and careful observations of yaws in its earlier as well as later post-frambesial stages, describe these conditions as belonging to yaws. Dartre and

pian gratelle especially are noted by them as being characteristic of yaws. In my own experience, psoriasis and lichen are just as frequent in occurrence in cases which develop frambesiae as in those which do not. The rheumatoid pains are almost always a feature of yaws, and these, as well as the less frequent palmar and plantar psoriasis and vesicular syphilides, are identical in both diseases.

In short, the *vox populi* has declared the truth, which the medical profession has not yet generally accepted, that yaws is non-venereal (or sometimes venereal) syphilis of the tropics. At any rate, the people name syphilitic manifestations and associate them with the frambesial eruption under the one name yaws.

A few recent writers on yaws, under its various names, have seen and recorded these facts; proving that yaws is more than the eruption of frambesiae, as described in text-books (e.g. Scheube). Even those writers who have not included these symptoms as part of the same disease have noted the frequency of the occurrence of syphilitic manifestations together with frambesiae. They regard the smaller syphilides as evidence of syphilis and reserve the frambesial eruption to be called yaws, and then speak of the concurrence of the two diseases. Daniels and Wallbridge note that "a fair number of such cases are "recorded, and in all the syphilis preceded the yaws."[1] It is doubtful whether by "syphilis" they refer to primary syphilis, i.e. the genital chancre from their point of view, or to small syphilides. Blanc, of Tobago, observes:—"I have not seen any yaws patients with syphilis "in the primary or early secondary stages, but have very commonly "seen them in the early stages of yaws with well-marked papular "eruptions, which, however, might have been either syphilitic or the "precursors of yaws tubercules. These symptoms commonly "disappear under treatment, but sometimes give rise to a scaly "eruption very like psoriasis. . . . Syphilis, as evinced by severe "cachexia, or indurated and enlarged glands, enlargements of bones, "nodes, &c., has been a very frequent complication in the cases of "yaws."[2]

There can be no doubt that the other secondary manifestations of yaws are identical with symptoms elsewhere ascribed to syphilis. Some writers attribute them to yaws, others to a concurrent syphilis.

1. J. Hutchinson, Fasciculus, p. 19.
2. Nicholls' Report, p. 165.

But anyone who cares to look for them will not have far to seek in almost any case of yaws.

Consistently with the description of the secondaries of yaws, Rat goes further and describes the tertiaries. In this he is in accord with Daniels and Corney in Fiji. These symptoms need no description—periostitic nodes, multiple dactylitis, destructive rhinopharyngitis, lupoid ulcerations, &c. It is only to be remarked that one does not realise to what extent tertiary syphilis can go until he has seen the cases of untreated syphilis in a yaws district.

It is evident that, in the experience of the writers just mentioned, there is not enough venereal syphilis to account for the extraordinary prevalence of tertiary, and so they were driven to ascribing the tertiary, quite correctly, to yaws. They have failed, no doubt, to see the non-venereal syphilis without frambesiae, which would have afforded an escape from the difficulty.

Tertiaries identical with those of syphilis are recorded as being exceedingly prevalent, and are attributed to yaws by Daniels and others in Fiji, Kynsey in Ceylon, Rat in Dominica. Among ninety cases of tertiary syphilis of whom the history was ascertained in St. Vincent, sixty-nine were said to have had yaws and only five admitted genital syphilis. Some of the remainder had suffered, no doubt, from extra-genital chancre without frambesiae, and therefore did not recognise that they had yaws. On the other hand, not all that claim to have had yaws have had a frambesial eruption, for, as I pointed out before, the natives, and apparently also some medical men, call cases yaws that have had no frambesiae.

From the facts of the polymorphism of the eruptions and the occurrence of tertiaries, it is evident that the only difficulty in the way of the general abandonment of the yaws heresy is the frambesial eruption itself. As Jonathan Hutchinson has pointed out, the profession did not originally hold yaws to be an independent disease, so that it is quite fair to speak of this belief that yaws is not syphilis as a heresy. The frambesial eruption is of such importance and, to my mind, such a formidable difficulty that I have reserved its consideration for another section.

My contention is that syphilis is very common, in fact, almost universal, among the natives in the tropics; that in certain districts it usually presents an eruption of papillomata, which has given rise to

the idea of yaws as a distinct disease; but that the cases with papillomata present otherwise all the features of syphilis, just as do the non-venereal cases. To appreciate the relation of the various forms of eruptions to each other and to recognise their nature, as well as to understand many other disease problems, it is necessary to remember that syphilis is in the tropics not usually a venereal disease. Notwithstanding the vitality of long-rooted error, I feel sure that the profession would have more readily accepted the dictum of Jonathan Hutchinson had we realised the frequency of extra-genital chancre. It is the teaching of the schools and text-books that syphilis is a venereal disease, which has blinded us to the fact that in the tropics it has little to do with sexual intercourse; that there is, in fact, far more non-venereal syphilis in the world than syphilis acquired on the genitals.

Case 14.—D. K.; 6 years; Coloured.

March 27th, 1906. Has had a sore of the toe and paronychia for several weeks. About two weeks ago began to get "spots" on the skin. Has now a few spots of psoriasis over the body and palms of the hands. Also on the knees and elbows. Is feverish at nights, has small hard glands in both groins and on elbows.

Here is a very early case in which, I think, the diagnosis of syphilis is justified.

Case 15.—J. W.; 13 years; Black.

August 1st, 1903. Has a small indolent dirty sore on the left outer malleolus, with raised edges and indurated base and periphery. This has been present for several weeks. There are tender glands in both groins. That on right larger and more tender. Small hard glands left side of neck, on both elbows and in both axillæ. Two weeks ago the first crop of eruptions appeared and they have continued to come out since. Has now a general papular eruption (lichen) tending to vesicular heads. This is very closely set over the whole trunk, extending to the neck, upper arms, and thighs, where it thins away. A few papules on the lower part of the cheeks, none on the limbs.

Also an early case, in which the diagnosis seems very evident.

Case 16.— R. B.; 10 years; Black.

April 6th, 1903. Three months ago had a scratch on right outer malleolus which became a sore. Her mother shows this as what she considered a mother yaw. It is now a pigmented and indurated scar. There are many spots of scaly psoriasis over the body, face and limbs—rheumatoid pains in the right elbow, both knees and ankles. The mother complains that the child is getting lazy and hard to rouse in the morning. Later she developed evening fever.

September 1st, 1903. Noted that she had attended irregularly. Eruptions are worse. Has never had frambesiae.

This illustrates the popular identification of syphilis with yaws.

CASE 17.—M. N.; 14 years; Coloured.

April 22nd, 1903. Has a small excavated ulcer about 6 mm. wide on a raised indurated base, on the outer side of the right foot. This has been present for several months. Hard enlarged glands in the right groin. Swollen tender glands in the right side of the neck. A chain of small tender glands in left side of neck. Fever, malaise.

A patch of superficial flat large papules with crusted scabs, each 10 mm. in diameter, on the front of the right thigh. These are like mild rupia or ecthyma.

CASE 18.—L. P.; 21 years; Black.

April 2nd, 1903. Has a deep excavated ulcer on the left outer malleolus. Does not admit venereal primary. Has no leucorrhoea to indicate cervical chancre. A coarse papular desquamating eruption of lichen on both hands and feet. Few papules on the face. Very profuse vesicular eruption on both legs and thighs. Glands enlarged in both groins and on both elbows.

In spite of the age of the patient, I had no hesitation in attributing her syphilis to infection on the ankle.

CASE 19.—S. N.; 30 years; Black.

March 15th, 1903. Has four small ulcers with indurated bases and raised, thickened, everted and irregular edges, round the right nipple. These appeared first three months ago while she was suckling an infant with yaws. They have all the appearance of primary chancres.

On 6th May, 1903. Complains of pain all over the body, malaise, and constipation. Looks and says she is very ill. Has nervous sensations and sleeplessness.

She never developed any eruption. Continued ailing and sickly for at least two years under treatment for which she attended irregularly, as she felt better or worse. Treated with small doses of mercury from the first. Never salivated. At length she complained mostly of gastric troubles. These were not due to mercury, for she only took doses of 10 minims of liq. hydr. perch. three times a day. After two years recovered health.

This was a case of visceral syphilis contracted from yaws. I have now under treatment a very severe case of gastric syphilis, in which the gastritis followed a year after a genital chancre. In this, also, there was no eruption, but adenitis and iritis.

CASE 20.—I. D.; 9 years; Black.

February 25th, 1904. A wide ulcer on the left outer malleolus, with a dirty yellow crust. Profuse patches of psoriasis and areas of lichen over legs, trunk and arms.

This would be called an early case of yaws, with the maman-pian, dartre and pian gratelle. By changing these names it becomes syphilis.

CASE 21.—G. P.; 9 years; Black.

1906. Small punched ulcer about 5 cm. wide on the back of the foot near the toes, with raised thickened edges. This has been present for several months.

There are hard glands in both groins, large soft glands in the neck. History of "glass-pox" in successive crops for several weeks, some months ago. Now comes complaining of fever, malaise, and anaemia.

In this case the knowledge of the occurrence of non-venereal syphilis led one to inquire about the eruption. This, together with the glands, the primary and the cachexia, leave no doubt as to the diagnosis. Chicken-pox, of course, does not continue in crops for several weeks.

CASE 22.—V. T.; 4 years; Black.

Mother was treated for a copious syphilitic rash eighteen months before. The patient was treated for an eruption some months ago. Now has a papular rash (lichen) over neck and shoulders. There are patches of weeping and exfoliating dermatitis over each side of forehead, on both scapulae, and on back of shoulders.

This is too old for hereditary syphilis. It is not unreasonable to attribute the rashes to syphilis acquired from the mother.

CASE 23.—R. S.; 5 years; Coloured.

1905. Multiple ecthymatous sores encrusted with yellow scabs on the legs. Large patch of the same on the left thigh, and a flat condylomatous spot with dry yellow crust among the ecthymata. Pigmented marks of large pustules about the body generally. Keratitis of one eye. Treated with mercury.

December 20th, 1905. Eruptions have disappeared, leaving no scars, but pigmented stains which subsequently faded. Admitted to Hospital for corneal ulcer and treated till well with mercury internally.

Here, as in the last case, there was no primary to be found, but the condition was evidently syphilitic, the age too late for secondaries of inherited taint. The eruption was one like frambesia, and even presented one spot of condyloma. The crusts, however, did not cover hypertrophied papillae, but eroded spots.

CASE 24.—M. D.; 3 years; Black. Mother seen on 28th October, 1902, with profuse eruptions of circular patches of scaly psoriasis over whole body, limbs and face.

January 5th, 1904. The child has a multiple eruption over both arms and legs, and back, of scaly psoriasis; patches of lichen in spots; and circinate and exfoliating dermatitis.

The age is too late for the secondaries of inherited syphilis; besides the mother has acquired syphilis only about fourteen months before. If the child had inherited syphilis from the father the mother would have been immune, by Colles' law. It is clearly an instance of acquisition from the mother.

CASE 25.—N. P.; 8 years; Black.

March 29th, 1903. Ulcer, 50 mm. wide on the dorsum of the left foot, which originated from a scratch three months ago. This has a raised fungating surface, and thickened raised everted edges. A very large gland in the left groin, large gland in right groin, gland in left elbow, and chains of shotty glands in the neck. Has intermittent fever, and constipation with dry hard stools. There is a desquamating condition of the skin of the extensor surfaces of forearms and legs, and a patch of coarse papules like common psoriasis on each elbow.

July 28th, 1903. Foot has healed. A flat yellow crusted eruption is on both knees and both elbows. Dry crusted warts on both hands.

This may almost be given as a case of yaws, except that the eruption was not perfectly frambesial. The patient lived in town; had he lived in a country village he would, no doubt, have had yaws.

These few cases, I think, illustrate the various aspects in which non-venereal syphilis may be presented to the physician: primary syphilis not followed by frambesia contracted from yaws; secondaries in children with and without an evident primary; secondary in an adult due to extra-genital infection. Two show the difficulty there may be in differentiating yaws. I selected five which were seen within five weeks at a time when my notes were being regularly kept. This number indicates how common such cases really are. (Cases 16, 17, 18, 19 and 25.)

ATTITUDE OF THE MEDICAL PROFESSION TOWARDS YAWS

In consideration of the symptoms of yaws, as referred to above, there would certainly never have been a doubt as to its identity with syphilis had it not been for the frambesial eruption. This is so unusual in European syphilis, and such a distinct and characteristic manifestation in yaws, that it was natural it should overshadow the other symptoms. Most physicians, who have not looked carefully into the matter, have a picture of yaws as consisting only of the frambesial eruption; they think this is a harmless, though unpleasant, disease, which usually runs its course and disappears spontaneously, without permanent injury to the health. But at first yaws was included with syphilis, and even since its separation as a disease *sui generis*, the doubt that it was correctly separated has existed.

In view of the enormous prevalence of yaws in the tropical world, this doubt gives an importance to the disease which, I think, has not been appreciated; for it is a most serious matter if syphilis, the most destructive and far-reaching in its effects of all diseases, is being allowed to ravage whole communities untreated and almost unnoticed.

When in recent years the syphilist, who for forty years has been the authority to the English-speaking profession, pronounces his unqualified opinion that syphilis is yaws, it is time that the world took up the matter seriously. In his preface to Numa Rat's work on yaws, Jonathan Hutchinson expressed this view, based, it appears, largely

on the evidence offered by Rat himself. Since then, after further investigation, he has delivered, so to speak, an *ex cathedrâ* opinion to the same effect in Fasciculus XIV of the new Sydenham Society's Atlas, and presented the evidence for his opinion. Notwithstanding, the medical profession, with few exceptions, are in the same attitude of indifference. The new student of tropical medicine will hardly get from his text-books any indication that there is any serious or reasonable doubt about the independence of yaws. The 1903 edition of Manson makes a misleading reference to Hutchinson as holding "that yaws is possibly syphilis modified." The English edition of Scheube, 1903, only refers to Hutchinson to misunderstand him in a similar way.

Somewhat in consequence of this, those who believe in the identity of syphilis and yaws are in a small minority. Not many take much account of yaws at all. Of those who believe in the independence of yaws, it is only the few enthusiasts who have studied the matter that think it a serious disease. It is not to be wondered at therefore, that in most places yaws is not treated at all. Trinidad, especially in the island of Tobago, is the only colony in which I have heard of any rational and systematic attempt to deal with the disease, but even there, because they do not realise its gravity and true nature, the measures taken by the medical officers must be incomplete. In some other places the yawsy are in effect regarded as social offenders, and forcibly removed to yaws hospitals for treatment. Such a course can never obtain the sympathy and support of the people whom it is meant to benefit.

In the treatment at all yaws asylums and dispensaries, as far as one can judge from the literature of the subject and the reports, the frambesial eruption is regarded as the disease. When this dries, the patient is said to be "cured"; when another crop appears this is called a relapse. All physicians that have given mercury find that the eruption disappears rapidly as a rule, and that it is apt to recur when the mercury is stopped. Some think that the mercury has a bad effect, and that the yaws should better be allowed to run its course. Certainly the frambesiae disappear under the mercurial treatment, and as certainly they sometimes recur. No one thinks he has "cured" a case of syphilis because a crop of secondaries has faded; nor does he stop mercury on that account; nor is he surprised if more

secondaries appear even while his patient is still taking mercury. When treating a case of syphilis, one is thinking not of the secondaries but of the tertiaries. The second stage will usually run its course and the patient get well in time without mercury. But it is, as a rule, only the mercury given early that saves the patient from a life of tertiaries later on.

If the papillomata are all that there is, then yaws is hardly worth much trouble; but the observations, especially of Numa Rat and Daniels, have established the fact that there is an aftermath of evils. They could not help but attribute to the effects of yaws the vast amount of exaggerated ulceration, periostitis, necrosis, and dactylitis which they saw around them. Numa Rat boldly calls these the tertiaries of yaws, though he still believes yaws to be distinct from syphilis. If the diseases are different, then yaws is the worse of the two, and it is the most terrible scourge from which the human race suffers.

If even yaws is not syphilis, then there is still abundant indication that the treatment is the same, and we should insist on the early and persevering use of mercury; for the time for treatment of tertiary syphilis is in the early secondary stage, and by inference the same must be true of yaws.

If, however, we decided to regard yaws as syphilis, its importance would be at once recognised by every member of the profession; and the people, educated in time to pay proper attention to the disease, as Europeans do to syphilis, would assist in the effort to limit its spread and cut short its dire results.

A systematic and conscientious treatment of the cases, and a persistent education of public opinion, would at length effect what all the yaws asylums, dispensaries and commissions have failed even to begin.

In view of the theory of Hutchinson that yaws is the original disease, we may abandon the name syphilis, which bears but an indifferent repute, and speak of yaws. Recognising its nature, we may teach the prophylaxis of syphilis under its various local names. The writer has already prepared, with this view, a primer of hygiene for West Indian negro children, at the request of the Board of Education of St. Vincent.

I said before, that it is only the frambesial eruption which constitutes the difficulty in accepting yaws as syphilis.

Manson says:—"I may mention the primary sore, the infection "of the foetus, the adenitis, the exanthem, the alopecia, the absence "of itching, the iritis, the affection of the permanent teeth, the bone "and eye affections, the congenital lesions, the polymorphism of the "eruptions, the nerve lesions, and the gummata of syphilis. All these "are wanting in yaws."[1]

To take these in detail:—

(1.) The primary sore.—There is, as we have seen above, no doubt at all about this. Numa Rat made this quite clear, and the experiments of Charlouis are convincing. In short, as Hutchinson says, Alford Nicholls is the only observer who disputes the fact that there is always a primary lesion in yaws. Careful examination and enquiry will elicit this quite as often as in cases of syphilis.

(2.) The infection of the foetus.—It would be quite as logical to deny the inheritance of syphilis. It is so rare for a child to be born with syphilitic eruptions that few of us have seen such a case. Many infants are seen with yaws at the age that they might have the secondaries of inherited disease. But when such cases occur, we are in the habit of inferring that the yaws is acquired. Inheritance has been demonstrated in at least two cases mentioned by Hutchinson. I have never myself seen an infant known to have hereditary syphilis develop a frambesial eruption; but all the infants I have seen thus have been at once put on mercury, and kept at it as long as I continued to see them. They get better, or, more usually, I hear nothing of them for a long time until the parent applies for a death certificate. It cannot certainly be said that inherited syphilis is not sometimes frambesial, and it is known that a child may inherit the yawsy taint and develop the frambesia in due time.[2]

(3.) The adenitis.—This is an almost invariable accompaniment of the yaws infection. The experiments of Charlouis are conclusive, besides the experience of actual examination of yaws patients.

(4.) The alopecia.—In all my experience of many hundreds of cases of negro syphilis (non-frambesial) I have only once seen alopecia, and in that case the diagnosis was at first obscure, but

1. Tropical Diseases, page 530.
2. Hutchinson Fasc., page 18.

afterwards cleared up. A physician of far longer experience than mine of the West Indies tells me he has never seen syphilitic alopecia in a black patient.

(5.) The absence of itching.—This is admitted to be by no means an absolute rule in syphilis. Nor is itching at all the rule in yaws. When we consider the irritation of the small flies around a yawsy patient, the admission of itching in some cases is not remarkable.

(6.) The exanthem.—It is difficult to understand which particular eruption is referred to. All or most of the syphilides have been seen in yaws.

(7.) The iritis.—This is distinctly uncommon in negro syphilis, and is a comparatively rare symptom anywhere. It is quite feasible to imagine that, where syphilis is profusely manifested on the skin, the deeper structures, particularly the nerve system and its appendages, may escape.

(8.) The affection of the permanent teeth.—This must be extremely rare, at any rate, among West Indian negroes. I cannot remember ever having seen notched incisors in a black person. Finucane and Corney, as mentioned by Hutchinson, report having seen such occasionally in Fiji, where syphilis is not admitted to be present. One has the impression that either the negro race is exempt from this affection, or that it is caused by mercury given in infancy. I very frequently look at patients' teeth to note the tolerance to mercury, and also to note the irregularities which constitute a stigma of degeneration.

(9.) The bone affections.—These have been described frequently as tertiaries of yaws. But the author has already denied them to us by saying that they are due to an independent intercurrent syphilis.

(10.) The congenital lesion.—There are none in syphilis, except the rare pemphigus neonatorum, and the syphilitic causes of still-birth. No one has attempted to prove that miscarriage is any less frequent among yawsy mothers than among the syphilitic.

(11.) The polymorphism of the eruptions.—This is abundantly evident in yaws, and is referred to by Manson himself.

(12.) The nerve lesions and the gummata of syphilis.—The negro race is not prone to suffer from nerve syphilis, but all tertiaries and parasyphilis are taken from us by the simple assumption of no inter-current syphilis, of which there may be no other evidence.

As far, then, as all these differentia are concerned, it is not possible to separate the diseases. It is impossible to overlook the tertiary stage of yaws. The indications for treatment are the same as for syphilis. Even if the "syphilis theory" is not true, it is *as if true*, as Paul Carus would say; and, therefore, it is good to teach and useful to believe. Any abstruse difference based upon the frambesial eruption can only have an academic interest.

THE FRAMBESIA OR PAPILLOMATOUS SYPHILIDE

The frambesia has been variously referred to by writers as a tubercle, a sore, granuloma, or a papule. No doubt, it is not of an invariable form, but none of these terms are correct. In every case the frambesia is raised, and consists of a closely-set group of hypertrophied papillae. The dermis is not ulcerated, for properly the eruption leaves no scar. The frambesia is sometimes naked and moist, but more usually it is covered by a yellow crust. In some instances, and especially when the eruption is old, the crust is dark or dirty brown, but typically it should be yellow. There may be a yellow secretion under the crust, and in the early and active stages this is the rule. When old and undergoing resolution the frambesia is dry, and the crust, if present, is more adherent. If the yaw has been naked, it becomes in the dry stage covered with a film.

Usually the eruption disappears by slow absorption, each yaw becoming imperceptibly smaller day by day. No doubt, yaws occasionally ulcerates by accident or by bad treatment, and leaves a true scar, but this is by no means its natural tendency.

As a rule, the frambesiae are scattered more or less profusely over the skin. The corners of the mouth, around the anus and on the genitals are the favourite sites. Typically each yaw is round or oval, and measures 8 to 15 mm. across. But they vary much in size, and may be much larger. They are sometimes arranged in a circinate manner, and by coalescence of contiguous frambesiae a large, irregular or horse-shoe form may be produced. It is, certainly, a very striking thing to witness the uniformity of the appearance of a number of cases. One can go to a country village in a yaws district, as in most parts of St. Vincent, and collect twenty children in five

minutes all showing the eruption in almost identical form. There is small wonder that the profession, having regard to this eruption alone, has almost generally fallen into the way of making it constitute a distinct disease.

Alford Nicholls, who has made a thorough anatomical examination by sections of the frambesial as well as of the papular and "macular" eruptions of yaws (fig. XIX, in his report), shows that the papule of pian gratelle consists of a few hypertrophied papillae infiltrated with small round cells, which are also accumulated in the underlying dermis; the corneous layer of the epidermis is undergoing overgrowth and exfoliation. This is identical with the structure of a papule of syphilitic lichen. (The frambesia is beautifully shown in section in fig. XXII.) It consists entirely of very much elongated and hypertrophied papillae, with a great infiltration of round cells. The epidermis is desquamated so that the ends of the papillae are covered only by the secretion and the crust, which is formed of layers of dry secretion. The papillae are closely crowded together. This structure, without the crust, is identical with that of the syphilitic condyloma. The frambesia is, in fact, one of the large papules of syphilis. It is not, therefore, a granuloma in the sense that a gumma is. The small round cells are certainly the elements of granulation tissue, but the whole mass retains its physiological character as a group of natural papillae, which do not disappear so as to leave a scar. They have undergone an evolution and hypertrophy, and will return to their normal size by involution and absorption of the added elements. The term granuloma is a misleading one. The red granular surface of the frambesia, seen after the removal of the crust, to which it owes its name, from the resemblance to a raspberry, is not due to granulations such as are on an ulcer.

The proper pathological description of the frambesia is in the term papilloma; and it is structurally identical with the papillomata of syphilis, condylomata and rhagades. In a drying papilloma the crust fits into the interstices between the papillae, so as to be difficult to remove. But when it is removed, the individuality and tough consistence of the papillae can be easily demonstrated.

On moist surfaces, as on the vulva and around the arms, and sometimes at the webs of the fingers and toes, the papilloma is naked. The ends of the papillae are swollen, and squeezed together so as to

give a flat surface. Such frambesiae are indistinguishable from ordinary condylomata.

We have seen that it is only in the frambesial eruption that yaws differs from syphilis, but even the frambesia is identical with the syphilitic papilloma on a moist surface.

There is left, therefore, only the occurrence of condylomata all over the skin which can be held to be distinctive of yaws. Jonathan Hutchinson points out that a general papillomatous (frambesiform) eruption does occasionally occur in Europe. It has even been suggested that "Sibbens" was a syphilide of this form.

It is quite feasible to argue that on the thick, highly-developed and active skin of the dark races this exuberant form of eruption takes the place of the milder papules known in Europe. The higher infectiveness and greater prevalence of the frambesial syphilis is readily explained. Syphilis ordinarily (that is, as known among whites) is contracted from the primary and from moist papillomata-mucous plaques and condylomata. Yaws, however, has so many of the moist infective lesions on the surface of the body that contagion is far more easily effected under any conditions of life; and in the circumstances, and by reason of the habits of the natives, it must be almost impossible for each patient not to infect someone else. Infection can, of course, be effected venereally. Dr. Hatton, of Grenada, says, "I have known cases occur from sexual connection."[1] Dr. Boyd, of Grenada, reports a case, presumably of venereal infection of yaws:—"J. W.; a Creole. At 16 years, was perfectly healthy and "unexposed to any source of contagion, until deflowered by a man in "March, 1891. Fourteen days after this she found some tubercles on "the vulva and inside of the thigh, for which I admitted her to the "yaws hospital."[2] There is no suggestion that the man had yaws, and the appearance of a secondary on the inside of the thigh so soon after infection renders the case unreliable. Neither secondary syphilis nor yaws "tubercles" appear until some while after the primary. It is possible that the so-called tubercles were all small primary sores, those on the thighs being inoculated in finger-nail scratches received during the struggle.

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1. Nicholls' Report, p. 178.
2. Nicholls' Report, p. 198.

It is a fact that yaws is far more common than syphilis in the villages, and in towns *vice versâ*. When a medical man sees yaws in an adult, he never thinks of enquiring as to the possibility of its having been acquired venereally, and when he sees a case of ordinary syphilis he assumes the venereal infection, in spite, perhaps, of the asseverations and protests of the patient. In the country districts of yaws countries the syphilis, however acquired, probably for the most part shows frambesiae; while in the towns the non-venereal infection gives rise to the better-recognised forms of syphilis associated in our minds with venereal infection. Dr. Tulloch, of Tobago, remarks that the "primary sore . . . . is especially rare in the outlying districts where yaws is most common."

More observations are wanted on two points:—

(1.) Syphilis (without frambesiae) contracted from yaws.

(2.) Yaws (with frambesiae) contracted from ordinary syphilis.

As the date of infection will be three weeks or more, in both cases, before the first manifestation of the primary, and usually two or three months before the appearance of the secondaries, it will be extremely difficult to get histories, especially from ignorant peasants, to illustrate these two points. I have already described one instance of syphilis contracted from yaws (Case 19). Some cases are available of yaws supervening on venereal chancre, but the history of these does not include any proof of the nature of the disease from which the women suffered before they transmitted it as a venereal chancre.

I shall describe one such case. Similar cases, though not interpreted as I do, are reported by Numa Rat, W. Boyd and H. Bennett in the West Indies, and by Wallbridge in Fiji.

Rat's case is as follows:—"A man consulted the doctor about an "indurated sore of the prepuce, and had at the same time a small "ulcer on the ankle. During two months he was under mercurial "treatment, and had in this time sore throat, roseola, psoriasis and "enlarged glands. He then noticed a change in the ulcer; the "graulations became pale and large. Three weeks after this the "ulcer healed. A week, again, after this came a wide-spread crop of "papules. A few of these developed into frambesiae. The "'syphilitic roseola co-existed with the yaws papules, and the

"'syphilitic psoriasis with the yaws tubercles.'[1] A clearer case of "frambesial eruption supervening on venereal infection of syphilis "cannot be found."

W. Boyd's case is given as follows:—"A man had a well-marked "and indolent Hunterian chancre, and bubo, when he came under my "notice, and at the same time a mild eruption of frambesial tubercles, "which he stated made their appearance two weeks after the "syphilitic sore."[2]

H. Bennett's case:—"The patient was a young woman, who "contracted the syphilis first, and then got either a primary or a "secondary ulcer inoculated in an unknown manner with the yaws."[2] From the confidence with which the syphilis is spoken of, one presumes that a venereal chancre was known. I have, therefore, included the case here.

Dr. Wallbridge's case is quoted by J. Hutchinson[3]:—"The "patient, a white man, had syphilis, and developed frambesiae. The "latter disappeared on his return to England, and later, after he "again went to Fiji, he suffered with tertiaries."

In all these cases it is, of course, impossible to prove that the persons from whom the disease was contracted had syphilis of ordinary type.

There still remains one fact which forms the strongest objection to the identification of yaws with syphilis. This, so far as I know, has not been referred to by previous writers, and it remains for one, who for the last twelve years has been holding and teaching the syphilis theory, to raise what seems to be the strongest argument against that theory. Although it may be true that the nature of the dark skin predisposes to the frambesial eruption, it is indisputable that yaws is seen breeding true on white persons. In a yawsy village the poor whites get the disease and have the eruption just as certainly as do the blacks. On a white skin the frambesiae are less developed. The papilloma is not so prominent, and the crust less firm. That is, the yaws is flatter, moister, and usually smaller than on a black patient, and it is associated with more of other syphilides, psoriasis, large moist papules, ecthyma, &c. It is a condyloma

1. Nicholls' Report, p. 168.
2. Nicholls' Report, p. 166.
3. Fasciculus, p. 19.

of skin, nevertheless, and is of frequent occurrence; while in Europe such a lesion is so rare as to be a curiosity.

In considering a probable explanation of this phenomenon, we must note that even among negroes the frambesial eruption is rarely seen in towns, and while it is common in some West Indian islands, it is rare or absent from others.

I have already suggested that damp, hot places in the tropics, with dense vegetation, show a prevalence of yaws. This, I think, will be found a rule. Numa Rat states that yaws is absent from Anguilla,[1] which is flat, dry and barren. The typical yaws district is a deep valley or ravine in a tropical volcanic island, where vegetation is rank and the rainfall high.

Yaws is, therefore, very prevalent in Dominica, St. Vincent, St. Lucia and Grenada. It occurs to a less extent in Antigua, St. Kitts and Nevis. Antigua is flat and not wooded, except at one part. St. Kitts and Nevis, though lofty and volcanic, have long slopes sweeping away to the sea. The land here is cleared and cultivated. There are a few bushy ravines with villages, and in these yaws may be found.

There is, then, another factor besides the character of the skin which determines the appearance of frambesiae. We cannot yet predicate this factor, any more than we can explain many other examples of the variability of syphilis. Why, for instance, does lupoid ulceration of the face happen so frequently on the Windward side of St. Vincent, while destructive rhinopharyngitis is the rule on the Leeward side? Why do some patients get a formative periostitis, and others necrosis of bone? I may be allowed to copy from a recent letter of mine[2] :—"To understand this protean disease, "we must realise that various factors intervene to determine its "manifestations. We can appreciate some of these. The races and "individuals who tax their nerve system suffer from nerve syphilis, "which is comparatively rare among negroes. The negro, with his "highly-specialised and active skin, displays an exuberance of "eruptions rarely seen in Europe. The labourer, exposed to injuries, "develops grave bone lesions. The women in St. Kitts, who

1. Report on Anguilla. Colonial Reprints, No. 20.
2. Journal of Tropical Medicine, May 15th, 1906.

" habitually gratify the desire of sailors for paedicatio mulierum, get " stricture of the rectum. Some of the determining factors we have " no knowledge of, others we can now guess at."

The frambesial eruption, therefore, though a striking and characteristic feature of yaws, we have seen is not unknown in syphilis. The difference between yaws and syphilis is only in the frequency of occurrence of this eruption. If all that is called so is yaws, it must be admitted that the frambesia is not even an essential of yaws. In face of all the other points of identification, it is impossible to give this one feature such pre-eminence as to exclude the others. We must accept the papillomatous eruption only as a phenomenon of syphilis, of which there are many others still awaiting explanation. The enquiry into the reason of the frambesial eruption may well produce a practical result, for if it were possible to prevent syphilis taking this form, we should thereby materially limit its infectiveness, and so lessen the amount of syphilis in the world. Here, then, is the field for research into yaws, which may yield a vast store of health for the unhappy natives of tropical lands.

Syphilis as yaws is almost general in tropical countries wherever the conditions of damp, heat and dense vegetation obtain. It is recognised that coko in Fiji, puru in the Malay Peninsula, parangi in Ceylon, galtoo in the West Coast of Africa, tomo in Samoa, are all identical with yaws in the West Indies. Yaws is well-enough known in every tropical country to bear a local name, and the synonyms are as numerous as the languages of the peoples affected. It was endemic in all parts of the tropical world before the European appeared on these scenes to confuse the geographical distribution of races and diseases by his facilities for inter-communication. Syphilis in all forms is now universal, and was probably so from very ancient times. Its wide spread proves, I think, that it must have been well established at an early period before the differentiation and distribution of human races.

It has been suggested by Hutchinson that yaws was the original disease prevalent in the tropics, and that it was introduced into Europe from them. Under the influence of a different climate, and the cleaner habits of the Europeans, it was limited and modified until it became usually venereal. He thinks that Sibbens and Button-scurvy were frambesial syphilis. Considering the wide

distribution of yaws, it is unlikely that the European countries could have escaped infection until modern times. The forefathers of the Caucasic peoples must have been already infected before they left the race-home on the pleistocene plateau of Sahara.

The experiments of Metchnikoff and Roux have shown that apes can be inoculated with syphilis. The femur of *Pithecanthropus erectus* shows evidences of disease very suggestive of syphilis. I am aware that these have been attributed to myositis ossificans, but one would think that the latter is more likely to be a recent disease resulting from the artificial circumstances of human life. In the Chaldean epic of Izduhar or Gilgames, it is told how the demi-god, having incurred the anger of Istar, was afflicted with a plague. An eruption of sores covered his body, his bones ached, his strength waned, his hair fell out. At length, under the advice of his beatified ancestor, Hasisadra, he was restored to health by sea-bathing. George Smith and other translators of the cuneiform script, following him, have read this to be leprosy. But every eruption on the skin is loosely so called by those writing on ancient matters. The leprosy of the Levitical regulations (Lev., chapters 13 and 14) was certainly not lepra. It no doubt included more than one disease, but most likely it referred chiefly to yaws. There is, perhaps, a reference to psoriasis in the " bright spots," and to frambesia in the appearance of the raw quick flesh. The disease was one of rapid development, for a suspect was kept under observation for seven days, and then for seven days more. Provision is made for restoring the outcast patient to society when he became clean. This would have been useless in the case of leprosy. The suggestion that Leviticus refers to yaws is a very old one, and has been discussed unfavourably by Alford Nicholls. But there is no common disease except syphilis which would explain all the regulations. True leprosy, no doubt, came under the operation of the laws, and was dealt with along with the syphilis.

It may be claimed that the symptoms of syphilis were sufficiently well known to find a place in the folklore of the early Semitic tribes, and to be subsequently included by the compilers of the traditional history and the authors of the great poems; and it was found necessary to devise an elaborate legislation to prevent its spread. The epic of Gilgames cannot be dated earlier than the second

millennium B.C. It has already enveloped in myth an invasion of the Elamites, which occurred about 2300 B.C. But even that is a respectable antiquity, and if syphilis was known to the poet of this the oldest epic in existence, there has been ample time and opportunity for it to spread to Europe before the middle of the second millennium A.D.

It is now known that endemic non-venereal syphilis may flourish even in Europe. Metchnikoff[1] points out that in rural districts in Russia the children are the chief sufferers and agents of the spread of syphilis, just as they are of yaws in the tropics. There is no reason for postponing the introduction of the disease into Europe to the 16th century, when it is far more probable that it has been present and spreading in a non-venereal manner as long as the human race has been in existence.

I append a few cases of yaws, that is, cases that displayed the frambesial eruption alone or, as is more usual, accompanied by other syphilides.

CASE 26.—C. W.; 8 years; Black.

January 23rd, 1906. Seen with an ulcer at the web of toes.

February 10th, 1906. Complained of pains, fever, and malaise, given liq. hydrag. perch.

March 24th, 1906. Onychia of nearly all the toes, whole body spotted with small round patches of scaly psoriasis. A large frambesia at the arms, which is said to have appeared a week before. Large glands in both groins. Ulcer on the plantar surface of web of toes is still in the same state as when first seen. Took six weekly injections of salalembroth. The frambesia disappeared rapidly, the skin cleared and the foot healed.

Discharged from treatment with directions to return in a month. Has not been seen since.

CASE 27.—C. B.; 3 years; Black.

March 1st, 1906. Has had yaws lately. One small frambesia still apparent at the angle of mouth. Had glands on elbows, in back of neck, axillae, below both angles of jaw, and in groins. Profuse eruption of small raw eroded surfaces, not raised but covered with moist yellow pellicles, in the left axilla. Few similar, drier and more crusted in the right axilla. Two such on the abdomen. Papules of similar character, some moist and some crusted, on and behind both auricles.

CASE 28.—M. P.; 8 years; Black.

March 7th, 1903. Had a sore on right ankle some months before. Large gland in right groin. Small hard glands on both elbows and many in the neck. Complains of pain in knees and ankles. Several marks of previous yaws on the legs. Several frambesiae with crusts on the right knee and the back. Papular eruption (lichen) between shoulder blades and round the axillae.

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1. Harben Lectures, 1906.

CASE 29.—M. J.; 12 years; Black.

January 16th, 1903. Numerous small, perfectly-smooth limpets, which when removed are horny cups covering a granular raised papilloma identical with frambesia. These are over the face, hands, and body. They are about 6 to 10 mm. in size. A few of the papillomata have yellow crusts as is usual for frambesiae. On the left ankle is a spreading patch of a mildly lupoid appearance, the edge being active, and in the centre of this is an ordinary frambesia. This is probably the mother yaw. Treated with liq. hydrarg. perch.

February 19th, 1903. The limpets and horns are larger.

February 12th, 1904. A small rupia over left eyebrow, small tubboes on both soles. Circular wall of rupial scab on both hands at the base of the fourth and fifth fingers. Rhagades between the fingers of the left hand. No marks of the previous limpet eruption. This exemplifies a dry type of papilloma, and also shows later secondaries of rupial type.

CASE 30.—F. C.; age not noted; Black.

December 30th, 1902. Has a dry crusted frambesia on the wrist. Suppurative dermatitis of both palms and both soles. Multiple small pigmented marks as of a vesicular or pustular eruption on both legs. Psoriasis on both elbows and knees. Took pot. iod. for five weeks and left off attending.

I have seen papular groups, without scaliness, on the knees and elbows in syphilitic cases so often, that I doubt the opinion that psoriasis in this situation is never syphilitic.

September 14th, 1903. Tubboes on soles, and plantar and palmar psoriasis (crab yaws), keratitis. Voice raucous. This illustrates the polymorphism of the eruptions and the symmetry so characteristic of syphilis.

CASE 31.—C. B.; 30 years; White.

January 2nd, 1906. Has a child with yaws. Ulcer on heel about 20 mm. wide, with thickened raised edges, and ichorous discharge. This began as a pimple two months before. The first eruption was that now on the same foot and lower leg, which is a moist dermatitis. There are crusted ecthymata and aciniform eruptions all over the body and sparsely on the limbs and face. Slight fever every evening. Urine high coloured. Rheumatoid pains in shoulders. Paronychia of one finger. No enlarged glands. Given liq. hydrag. perch.

February 13th, 1906. Profuse small spots of silvery scaly psoriasis on both arms and thighs. In the bend of right elbow are three drying frambesiae. Several frambesiae round the trunk, and a profuse eruption of them on the left foot. Few on the right leg. Groin glands enlarged. No fever pains or malaise. All frambesiae are small and not much raised. Received a course of seven injections of salalembroth during which the smaller eruptions faded, but frambesiae continued to appear. Was rested for a month from mercury. At the end of this time the skin was nearly clean and only a few frambesiae left. Continued on liq. hydrag. perch.

CASE 32.—M. P.; 8 years; Black.

1906. Sent to hospital as a case of syphilis by a medical officer who had just come out. Frambesiae on two toes. Condyloma of anus. Condylomata of prepuce and circinate plaques on the glans penis. Enlarged groin glands. Circumcised and treated with six injections of salalembroth. Quite clean, and discharged.

CASE 33.—A. W.; 9 years; Black.

1906. Two large frambesiae by the side of a scabbed sore on the back of the left foot; and whole body, limbs and face covered with spots of psoriasis, even the palms of the hands. Hard glands in both groins, left being especially large. Glands on elbow.

Case 34.—Female; 7 years; Black.

1906. Large irregular branched ulcer on a thickened base and with raised edges, on the back of the left heel. Glands in both groins and shotty glands in neck.

Several frambesiae on cheek and neck. Very large frambesiae at side and back of one knee, dry crusted papillomata on forehead and back of neck. These are essentially the same as yaws, but are dry, and have dark scabs. A symmetrical lichen covers both buttocks and back of thighs.

Case 35.—L. K.; 19 years; Coloured.

April, 1906.—Had a venereal chancre last year accompanied by fever and rheumatoid pains. Glands in both groins enlarged. Profuse eruptions of frambesiform syphilides over both knees, extending to front of thighs and legs. These are about 12 mm. each. Some are moist with yellow pellicles, under which is a granular surface of elongated papillae. Some have dry brown crusts, which when removed expose a frambesial surface.

There are pigmented pits of a recent vesicular eruption on the right arm and the thighs.

The eruption in this case would have been called yaws by anyone. It was atypical in two respects. It was quite symmetrical, and the frambesiae were crowded together so as to be sometimes confluent. But neither of these features are exclusive of yaws. Such cases of yaws following a venereal infection are, perhaps, more common than is known. A syphilitic history is rarely enquired into when a patient displays yaws.

Case 36.—M. W.; 6 years, Black.

July 16th, 1906. Oval, flat, callous ulcer, with a glazed surface and pigmented areola, on the front of the left shin. Enlarged glands in both groins and both elbows, several large glands in the neck.

A crop of small frambesiae with dirty-brown crusts on the forehead. Several larger flat condylomata with slight crusts, or naked with moist yellow surface, on the front of the neck and right cheek. Numerous papules varying in size scattered on the face. On the left cheek a group larger, and almost frambesiform. Small frambesia on the vulva. A profuse eruption of dry papules of all sizes up to 4 or 5 mm. over both buttocks and back of thighs. On the elbows a warty eruption of large papules with hard dry horny tops. Many of the papules elsewhere have a horny plug or horny apex of epithelium. There is a fine papular eruption on the front of the knees. Few scattered papules all over the skin. This case well exemplifies the polymorphism of the yaws eruptions. There were at least five syphilides of different appearance present at once.

TREATMENT OF SYPHILIS IN NEGROES

If we decide to treat yaws, whether it be syphilis or not, with mercury, it is necessary to appreciate that the treatment must be persevered in for many months. The frequent experience of the recurrence of frambesial eruptions after being presumably "cured" by mercury, indicates that the mercury must be continued, as in syphilis,

more or less through the first two years off and on. It will be found a very difficult thing to get a peasant to understand or act up to this, which in most cases will be only a counsel of perfection. When it comes to the tertiaries and potassium iodide, the patients readily learn from the relief obtained to value the drug. In St. Kitts and in Fiji the natives are fully alive to the uses of iodide, and prescribe it for themselves freely.

I have noticed doses as high as ninety minims of liq. hydrag. perch. mentioned in some of the literature on yaws, and there is very frequent reference to salivation. I wish to draw attention to the fact that negroes bear mercury badly. My father, a keen syphilist with many years' experience of treating negroes in hospital, where one can see best what one is doing, warned me of this. Fifteen minims three times a day is, I believe, about his limit. My own experience has amply borne out this. Though one is not afraid of "touching" the gums of a patient, and, in fact, generally likes to see them just "touched," as an indication that mercury is being pushed to its limit, yet salivation is undesirable. Apart from any physical effect it may have on the health or strength of the patient, it is apt to shake his confidence, and perhaps frighten him away altogether. It is astonishing how quickly some blacks do become salivated. I have seen a woman very badly ptyalised by five grains of calomel. Blue ointment applied to a wrist for a few days salivated a man, and a young woman became salivated before she had finished using half an ounce of blue ointment by daily inunction of about one dram. In the last case, there was certainly no idiosyncrasy; she had taken mercury, by the mouth in small doses, freely before, and has since received a course of intramuscular injection of 0·03 gme of salalembroth weekly, equivalent to about $\frac{2}{7}$ gr. of corrosive sublimate.

I have used mercury by inunction of blue ointment, mouth administration of liq. hydrarg. perch., calomel, and grey powder, and intramuscular injections of salalembroth. After a fairly large experience, I am confident that mercury is borne best when given by the last method. Inunction may salivate quickly and suddenly. In hospital one can watch it, but even so the ptyalism may occur suddenly. Grey powder with carbonate of iron is an excellent treatment, especially for children with hereditary taint or yaws. It

is convenient also for adults, for they can be given a hundred pills at a time, which last a month. I do not like to exceed 0·1 gm. thrice a day. Calomel I do not value for antisyphilitic treatment; but for general use in dispensary work the liq. hydrarg. perch. in a mixture is the most useful. I generally colour this lightly with methyl blue, which gives an impressive and distinctive value to the bottle. One should begin at the rate of 2 c.cm. of liq. hydrag. perch. per day—say, 10 minims three times a day, and increase to 3 c.cm. It is hardly necessary to go higher than this. Children bear this and any mercury well in proportion to the adults. For an infant of three months with inherited disease I give 0·15 c.cm. three times a day, equal to about two and a half minims.

Intramuscular injection of soluble salts is, however, far the best mode of administering mercury in my experience. I cannot speak of the insoluble preparations, for, being quite satisfied with the salalem-broth, I have not tried the emulsion of metallic mercury so highly spoken of by some. In spite of the pain, I have found that out-patients attend very well. Out of a number who were to take six injections each, the average attendance was five times. I have used this method on infants with hereditary syphilis also, and in yaws. My experience is not yet very large, but I have had abundant opportunity of proving that some negroes can carry up to 1·5 c.cm. of a 2 per cent. solution of mercury perchloride without any sign of spongy gums. This is equivalent to nearly half a grain of the salt, or about half an ounce of liq. hydrag. perch. Usually, I begin with 0·7 c.cm. and work up to 1·0, using a solution containing mercury perchloride 2 per cent., ammonium chloride 1 per cent, and giving one injection weekly. With care, washing the buttock with an antiseptic and then with ether, I have not yet seen an abscess result, and only rarely any induration. For hospital treatment, or for regular yaws dispensaries, the intramuscular method should be the most certain, the easiest and most effectual method of giving mercury. The treatment is shorter, and for that reason more useful in the case of natives. It should be possible to get most cases to attend for six or eight weeks for a course of injections, and repeat this twice more within the first year. After this, one attendance a month for another six months. The symptoms that may be expected should be explained to the patient, in order that he may at once report himself on the occurrence of any of them; and

certainly it is our duty to enlarge to any extent on the terrors of the tertiaries.

There is no necessity for special yaws asylums, but it is very desirable to have some provision for hospital treatment of severe and cachetic cases. Special wards in a general hospital would meet the requirements.

In the local treatment of yaws, I have obtained best results from a paint of zinc oxide and calamine, an old-fashioned but ever-useful preparation. It is readily applied by the patient or his parents; it is not easily wiped off, and, most important of all, it keeps the frambesia dry. I feel confident that a case of yaws is not very likely to infect other persons from his skin lesions as long as the frambesiae are kept dry and well coated with this paint. The risk of tonsillar infection from spoons and cups cannot, of course, be avoided in the peasant class, and that this is a frequent mode of infection with syphilis is well recognised.[1] But the most important material of infection in yaws is the secretion of the frambesia, and this can be limited by local applications, and by causing the absorption of the papilloma as rapidly as possible with mercury internally.

1. Kyle, Diseases of the Throat, p. 464.

A DESCRIPTION OF SOME GOLD COAST ENTOMOSTRACA

A DESCRIPTION OF SOME GOLD COAST ENTOMOSTRACA*

BY
W. M. GRAHAM, B.A., M.B.

It is a matter of common observation in the Gold Coast Colony, Ashanti and the Northern Territories that the incidence of Guinea worm varies locally. Some stations shew a high percentage of cases, some a low percentage, and some are free from the disease. Why should such local variations exist? The probable explanation is the abundance, the variety or the absence of the Cyclops-host from the water sources of the locality. Cyclops belongs to a widely-distributed family, and some species of the family can be found readily in the water sources of every station. But as Guinea worm disease is not present at every station, it is evident that the presence of any species in the water is not sufficient to cause the disease. The inference seems probable that a special species of Cyclops is required to act as efficient host to the worm, and that the absence of such species from the water sources of a station coincides with the absence of Guinea worm disease. If this be true, then an exact knowledge of the species to be found at each station becomes of great practical importance, but there is at present no classification of Gold Coast Copepoda. I have, therefore, sought in the present article to furnish means for the identification of the species found in the water sources of the station of Obuasi, and in those of the country within a three-mile radius round it, as a first instalment. Obuasi is a mining village on the Gold Coast Government Railway, 124 miles from Sekondi. The country is hilly and covered with forest. During the dry season the streams are small, sluggish and full of weed. With the setting in of the heavy rains they become rapid torrents, and the weed disappears. During the first period, to the end of April, Cyclops 1 to 5 can be caught in the streams, but after the first week in May

* The thanks of the Editors are herewith given to Dr. Brady for his kindness in identifying and describing this collection of Entomostraca.—EDS.

I have failed to find them there. The country is covered with gold holes of varying depth, and along the railway by borrow-pits. The water in the shallower pits drys up, and by the end of the dry season the bottoms of the pits have been baked dust dry. After a week's heavy rain these pits begin to retain a permanent pool of water. Upon this pool frog spawn soon appears, and shortly afterwards adult females of No. 6 (*Cyclops leuckarti*, Claus) can be found. By the middle of May the intermediate nauplius forms have appeared. Then the females become gradually less numerous and males increase in number, until by the middle of July the females have become rarer than the males.

Meanwhile, Cyclops Nos. 2, 3, 4 and 5 appear in the pools in relatively small numbers, and are soon accompanied by their nauplius forms.

Lastly, Cyclops No. 7 (*C. simillimus*, sp. nov., Graham) appears in the pools (the females first, the males later), and seems to take the place vacated by *C. leuckarti*.

This account brings up the history to the end of July; and I was transferred early in August to another station.

The inference previously alluded to, that all species of Copepoda cannot act as efficient hosts to Guinea worm, is strengthened by the following considerations:—

1st.—There is a large number of species.

2nd.—The habits of the different species vary greatly. Some are surface feeders, some are found at the bottom. Some inhabit foul, some clean water. Some leave the water to climb on stalks of water-weed enveloped in a drop of water carried with them, some do not leave the water. Some are found in streams, some are not.

3rd.—The different species are infested by different parasites. I have found Nos. 3, 4 and 5 infested only by ecto-parasites (algae), and No. 6 infested by ento-parasites (worms).

4th.—The different species differ in the date of their appearance in the pools. Some are found early in May; some appear, or, at least, only became numerous, in July. The importance of this variation in date has been already explained by me in a former article (B.M.J., 11/11/05).

A careful examination of the water sources of each station, and a classification of the Copepoda found in each place, is urgently required. When it has been made, a comparison of the species of Cyclops found at a station where Guinea worm is common with the species of Cyclops found at a station where Guinea worm disease is absent would, I have no doubt, offer an explanation of the observed local variation in the incidence of the disease. The practical value of an explanation is evident. It would enable the Medical Officer to identify those water sources likely to aid in the propagation of the malady.

CYCLOPS NO. 1. ? *Cyclops bicolor*, G. O. Sars.

MALE.—Colour yellowish, with darker coloured first antennae. First antennae, 11 segments as long as $\frac{3}{4}$ cephalothorax. Total length, 0·46 mm. Cephalothorax and thorax, 0·30 mm. Furca, 0·03 mm.

FEMALE.—Colour as in male. First antennae as in male, but more slender; total length, 0·62 mm. Cephalothorax and thorax, 0·42 mm. Furca, 0·04 mm. Egg sacs, a pinkish colour, carried apart.

In both series the outward tail sita is long. Found in pool with Calanus No. 1; water clear, and used by village as supply.

CYCLOPS NO. 2. *Cyclops varicoides*, sp. nov. (Brady)

MALE.—Colour, transparent pale yellow. First antennae, 12 segments, nearly as long as cephalothorax. Total length, 0·59 mm. Cephalothorax and thorax, 0·38 mm. Furca, 0·04 mm.

FEMALE.—Colour as in male. First antennae as above, but more slender. Total length, 0·64 mm. Cephalothorax and thorax, 0·39 mm. Furca, 0·04 mm. Egg sacs, a yellow colour, carried close together.

Found in river water.

CYCLOPS NO. 3. *Cyclops longistylis*, sp. nov. (Brady)

MALE.—Colour, a pale yellowish-green. First antennae, 12 segments as long as cephalothorax and two thoracic segments. Total length, 0·70 mm. Cephalothorax and thorax, 0·45 mm. Furca, 0·10 mm.

FEMALE.—Colour as above. First antennae as above, but slender. Total length, 0·84 mm. Cephalothorax and thorax, 0·50 mm. Furca, 0·12 mm. Egg sacs, a violet colour, carried slightly apart.

This species is frequently covered partially or entirely by ecto-parasites (algae).

It can be readily distinguished from No. 2 by the great length of the furca.

CYCLOPS NO. 4. *Cyclops virescens*, sp. nov. (Brady)

MALE.—Colour, cephalothorax a pale yellow-green; thorax a dark green. First antennae, 10 segments; copper coloured, carried a sparkling spot below the eye when swimming; an air bubble in mouth. Total length, 0·47 mm. Cephalothorax and thorax, 0·29 mm. Furca, 0·03 mm.

FEMALE.—Colour as above. First antennae as above, but lighter in colour. Total length, 0·60 mm. Cephalothorax and thorax, 0·40 mm. Furca, 0·04 mm. Egg sacs, pale yellowish, carried very close together.

Caught in rapid streams, and later on in year in ponds.

CYCLOPS NO. 5. *Cyclops pheleratus*, Koch

MALE.—Colour, a bright copper. First antennae, 10 segments, as long as the cephalothorax only. Total length, 0·60 mm. Cephalothorax and thorax, 0·38 mm. Furca, 0·05 mm. Legs of a light blue colour.

FEMALE.—Colour as above. First antennae as above. Total length, 0·77 mm. Cephalothorax and thorax, 0·46 mm. Furca, 0.08 mm. Egg sacs, a bright blue colour, carried close together.

This species leaves the water readily, and climbs on the sides of the vessel carrying a drop of water with it.

CYCLOPS NO. 6. *Cyclops leuckarti*, Claus

MALE.—Colour, a very pale yellow-green. First antennae, 17 segments, as long as the cephalothorax and thorax. Total length, 0·89 mm. Cephalothorax and thorax, 0·54 mm. Furca, 0·06 mm.

Become plentiful at end of July. I have not found males before the middle of July.

FEMALE.—Colour as above, but not so very pale. First antennae as above. Total length, 1·04 mm. Cephalothorax and thorax, 0·65 mm. Furca, 0·10 mm. Egg sacs (white) transparent, a long oval, egg very circular, sacs carried at an angle of more than 45°.

Found in stagnant pools in May. Become scarce in July.

CYCLOPS NO. 7. *Cyclops simillimus*, sp. nov. (Graham)

MALE.—Colour, very pale salmon with orange spots (circular) in cephalothorax. First antennae, 17 segments, somewhat longer than cephalothorax and thorax. Total length, 0·62 mm. Cephalothorax and thorax, 0·35 mm. Furca, 0·05 mm.

FEMALE.—Colour, pale yellow-green with circular orange spots in cephalothorax. First antennae as above, a very pale yellow. Length as above. Total length, 0·80 mm. Cephalothorax and thorax, 0·49 mm. Furca, 0·05 mm. Egg sacs, a pale yellow, carried apart.

This species also usually carries an air bubble in mouth when swimming. It differs from No. 6, being smaller and having relatively shorter tail setae.

CYCLOPS NO. 8. (*non det.*)

MALE.—Not found.

FEMALE.—Colour transparent, with slightly milky spots in cephalothorax and thorax. First antennae, 17 segments, as long as cephalothorax and thorax. Total length, 0·79 mm. Cephalothorax and thorax, 0·48 mm. Furca, 0·09 mm. Egg sacs small, milky white, carried apart.

Caught in well half a mile from the sea. Water in well clear, but slightly brackish. Frogs in well.

CALANUS NO. 1. = *Diaptomus innominatus*, sp. nov. (Brady)

MALE.—Colour, a very pale blue, the antennae being somewhat darker. First antennae, left 25 segments, right 22 segments, modified as clasper. Second antennae, 8 segments. Total length, 0·95 mm. Cephalothorax and thorax, 0·67 mm. Furca, 0·05 mm. Legs, central three pair a violet colour; fifth pair modified as claspers.

FEMALE.—Colour as above. First antennae, 25 segments. Second antennae, as above in male. Total length, 1·10 mm.

Cephalothorax and thorax, 0·75 mm. Furca, 0·07 mm. Egg sac, slightly blue, carried beneath.

When swimming appear transparent, with a dark blue longitudinal spot in about centre of cephalothorax and thorax.

I have only been able to find it in a single pond under trees, in which Cyclops No. 1 was also present.

CANTHOCAMPTUS NO. 1. = *Attheyella africana*, sp. nov. (Brady)

FEMALE.—Colour, a bright orange. First antennae, eight segments. Second antennae, four segments, forked. Total length, 0·35 mm. Cephalothorax and thorax, 0·17 mm. Egg sac nearly as long as body.

Very common in certain pools, where they occur in enormous numbers.

NOTES ON DR. GRAHAM'S COLLECTION OF CYCLOPIDAE FROM THE AFRICAN GOLD COAST

BY

G. STEWARDSON BRADY, M.D., LL.D., D.Sc., F.R.S.

(*Received May 14th, 1907*)

The following notes are intended merely as guides to the identification of species, and do not attempt a complete morphological account of the various forms. The brief descriptions are based upon the examination of females only, the males having been left out of the account.

In addition to the various species of *Cyclops*, the collection contained specimens of a minute Cyprid, a species of *Diaptomus*, and one called by Dr. Graham *Canthocamptus*, but which belongs to the nearly-related genus *Attheyella*. Both these last-named forms appear to be new, and I propose for them the names *Diaptomus innominatus* and *Attheyella africana*; they, however, need complete figures of structural detail, which at present I am unable to give.

GENUS CYCLOPS

No. 1. ? *Cyclops bicolor*, G. O. Sars.

No. 2. *Cyclops varicoides*, sp. nov.

Anterior antennae twelve-jointed, reaching to the third cephalothoracic segment, both branches of the first four pairs of swimming feet three-jointed, fifth pair rudimentary; caudal stylets short, about as long the last caudal segment. Length of body, exclusive of tail setae, 0·77 mm.

No. 3. *Cyclops longistylis*, sp. nov.

Anterior antennae twelve-jointed, reaching to the posterior border of the first cephalothoracic segment, both branches of the first four pairs of swimming feet three-jointed, fifth pair consisting of a

single small papilliform joint with two terminal setae; caudal stylets long and slender, about six times as long as broad, and equal in length to the three preceding caudal segments. Length of body, 0·78 mm.

No. 4. *Cyclops virescens*, sp. nov.

Anterior antennae ten-jointed, reaching to the third cephalothoracic segment, first four pairs of feet having both branches three-jointed, fifth pair minute, papilliform, bearing two long apical setae; caudal stylets short, about equal in length to the last caudal segment. Length of body, 0·6 mm.

This species is very similar to *C. gracilis*, Lilljebon, but differs in having all the branches of the swimming feet triarticulate; all the inner branches in *C. gracilis* being biarticulate; there are also other minor differences.

No. 5. *Cyclops phaleratus*, Koch

No. 6. *Cyclops leuckarti*, Claus.

No. 7. *Cyclops simillimus*, sp. nov.

Anterior antennae seventeen-jointed, reaching nearly to the posterior extremity of the cephalothorax, all branches of the swimming feet triarticulate, fifth pair biarticulate, last joint simple, narrow and bearing two long apical setae; caudal stylets about twice as long as broad, and nearly twice as long as the last caudal segment. Length, 0·77 mm.

EXPLANATION OF PLATE XXXIII

CYCLOPS BICOLOR, G. O. Sars

Fig. 1.—Female with ovisacs. × 74.
Fig. 2.—Male. × 74.

CYCLOPS VARICOIDES, n. sp., Brady

Fig. 3.—Female with ovisacs. × 74.
Fig. 4.—Male. × 74.

CYCLOPS LONGISTYLIS, n. sp., Brady

Fig. 5.—Female with ovisac. × 74.
Fig. 6.—Male. × 74.

The figures on this and the subsequent plates are reduced from Dr. Graham's original drawings.—EDS.

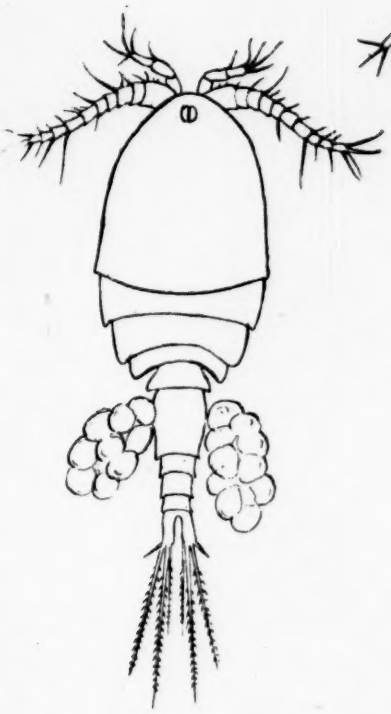

Fig 1

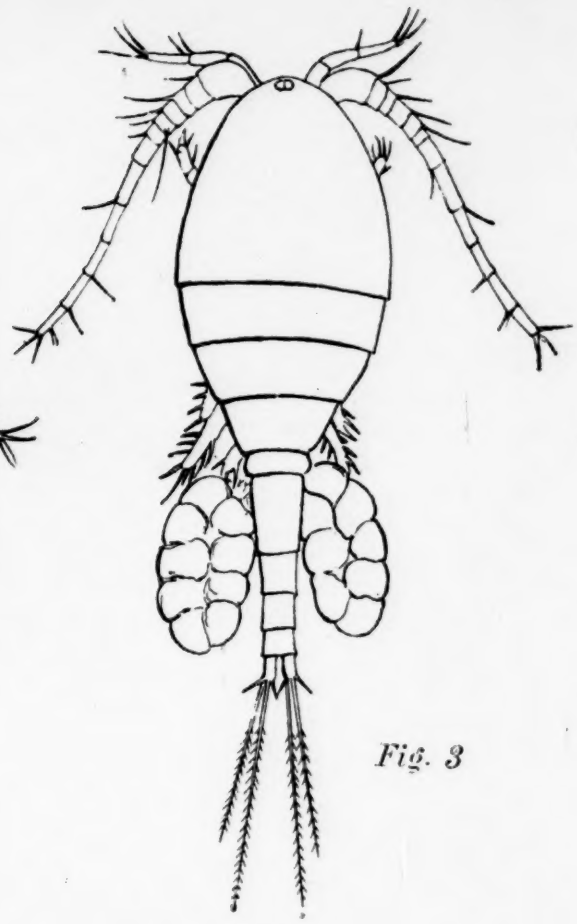

Fig. 3

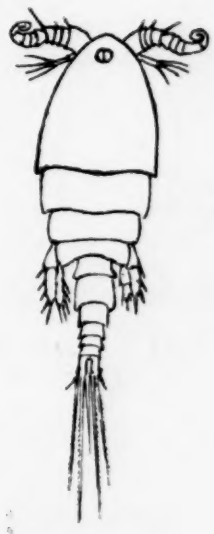

Fig. 2

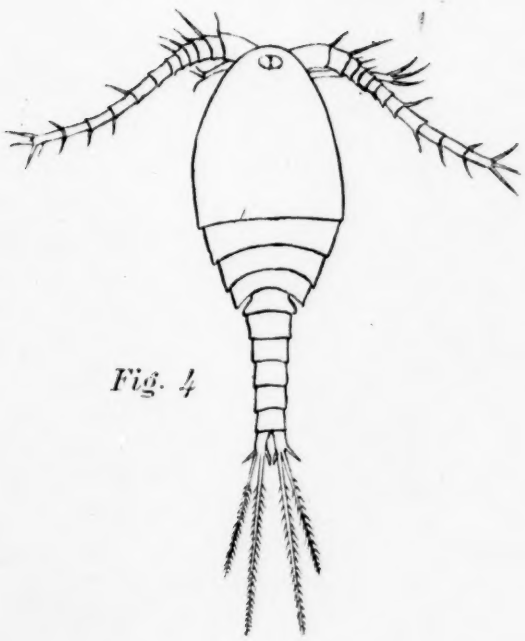

Fig. 4

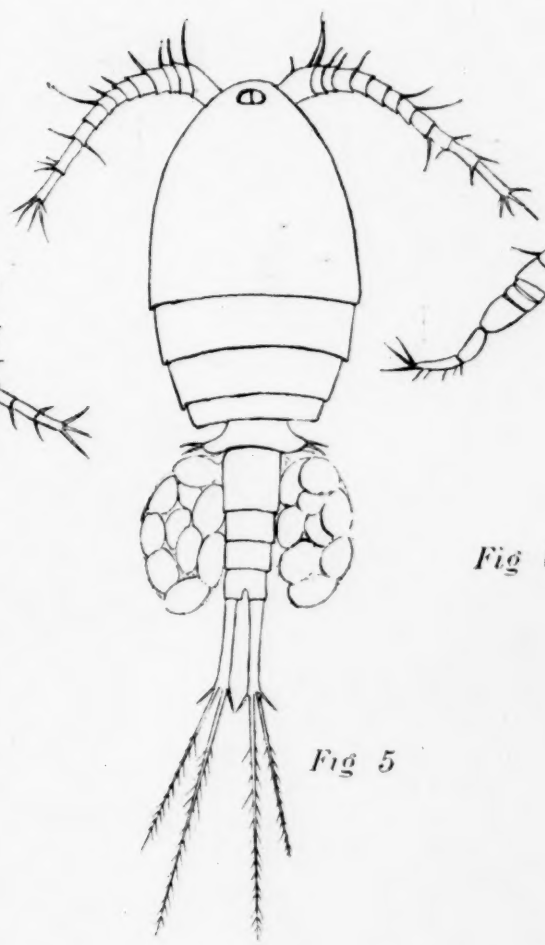

Fig 5

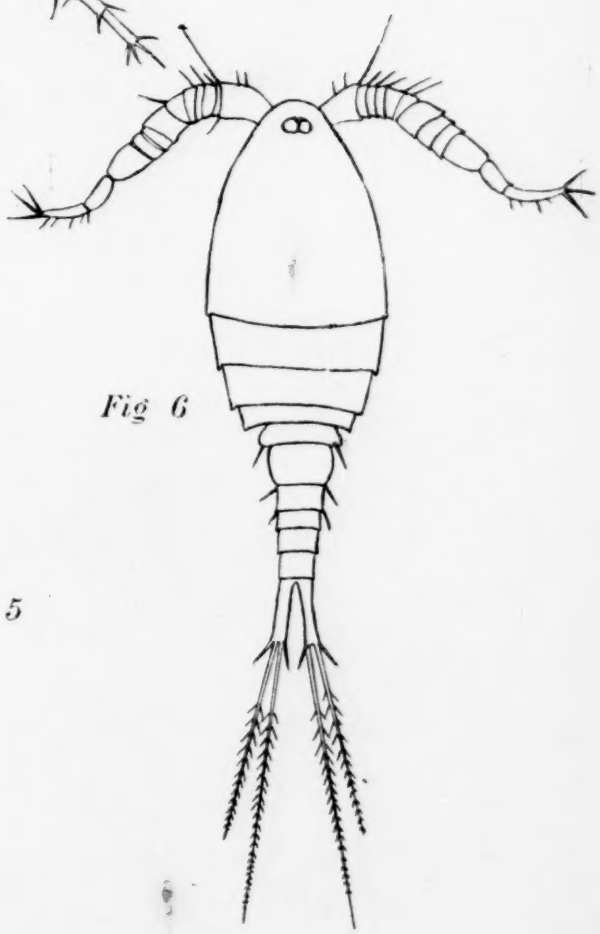

Fig 6

W. M. Graham, ad. nat. del.

EXPLANATION OF PLATE XXXIV

CYCLOPS VIRESCENS, sp. nov., Brady

Fig. 7.—Male. × 74.
Fig. 8.—Female with ovisacs. × 74.

CYCLOPS PHALERATUS, Koch

Fig. 9.—Female with ovisacs. × 74.
Fig. 10.—Immature. × 74.
Fig. 11.—Male. × 74.

CYCLOPS LEUCKARTI, Claus

Fig. 12.—Male. × 54.

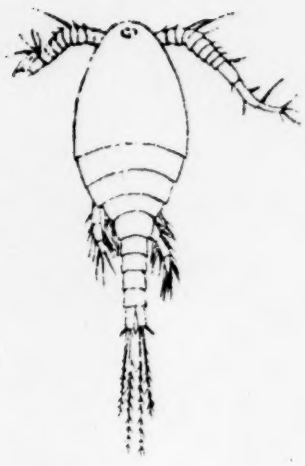

Fig 7

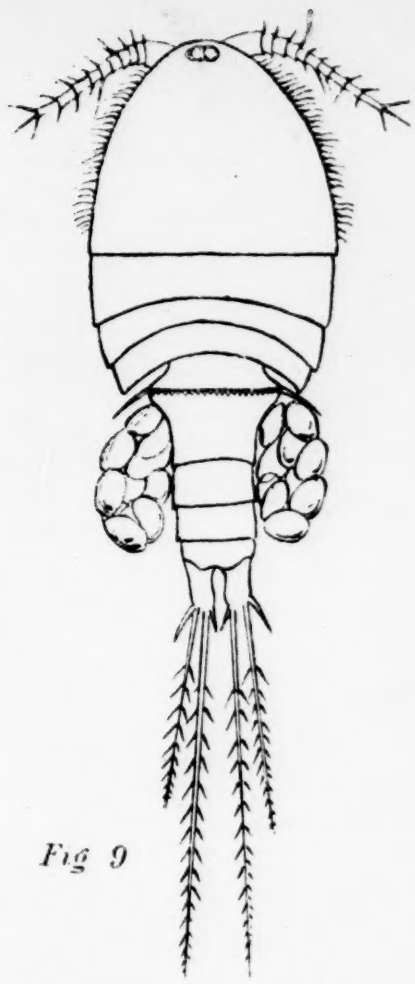

Fig 9

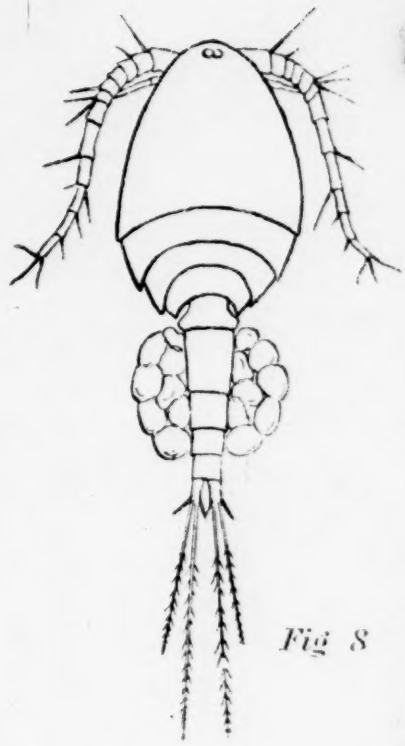

Fig 8

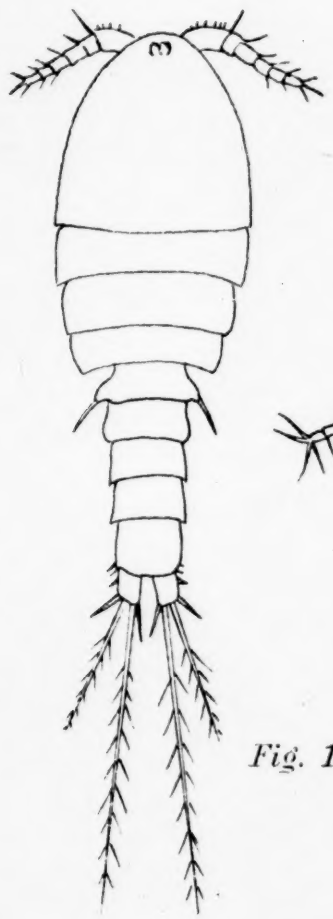

Fig. 10

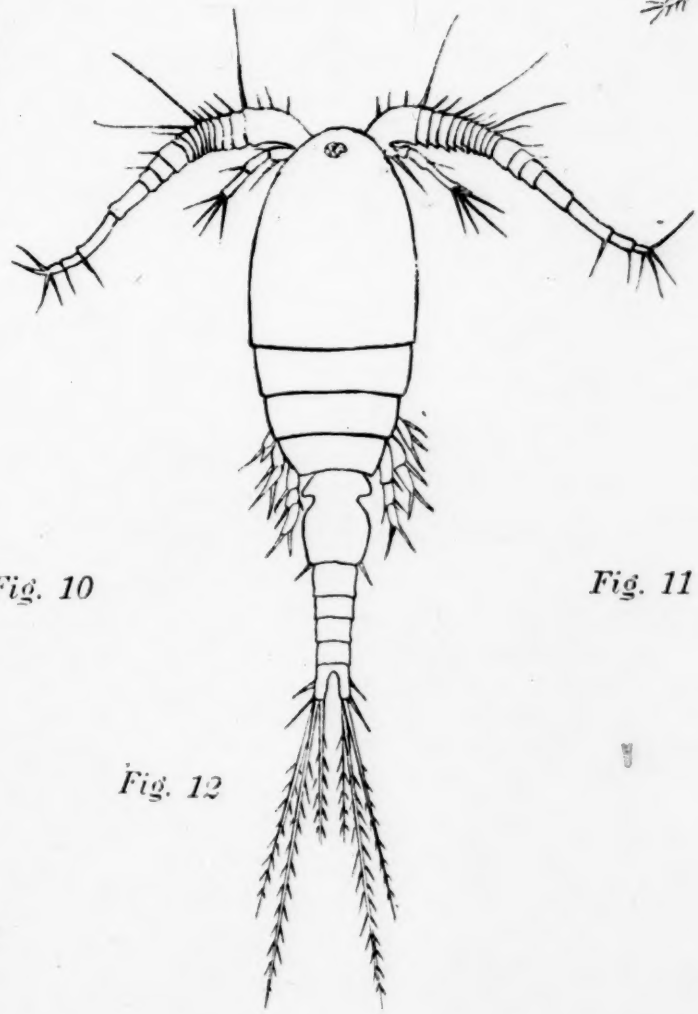

Fig. 12

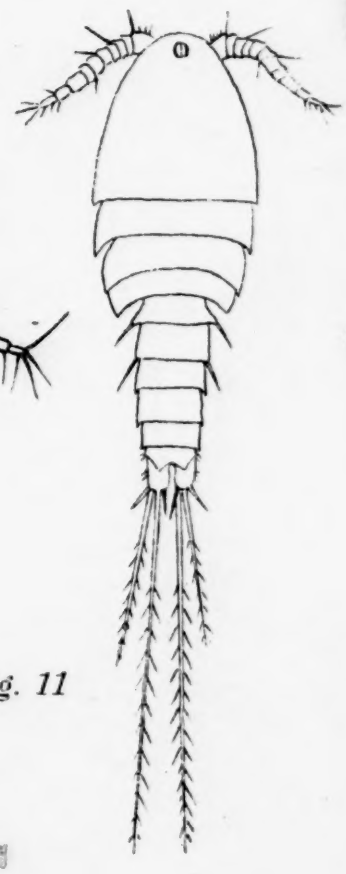

Fig. 11

W. M. Graham, ad. nat. del.

EXPLANATION OF PLATE XXXV

CYCLOPS LEUCKARTI, Claus

Fig. 13.—Female with ovisacs. × 74.
Fig. 14.—Immature. × 54.

CYCLOPS SIMILLIMUS, sp. nov., Brady

Fig. 15.—Male. × 74.
Fig. 16.—Female with ovisacs. × 74.

CYCLOPS, ? sp.

Fig. 17.—There were no specimens of this species in Dr. Graham's collection when submitted to Dr. Brady for determination.

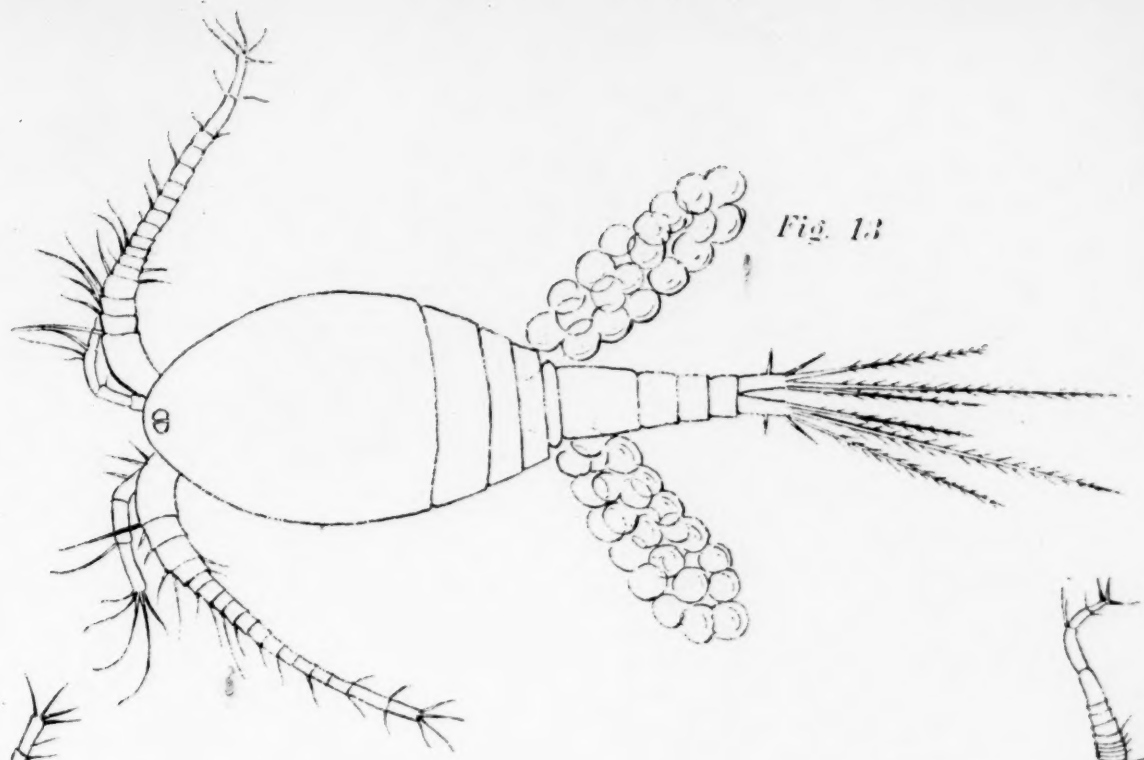

Fig. 13

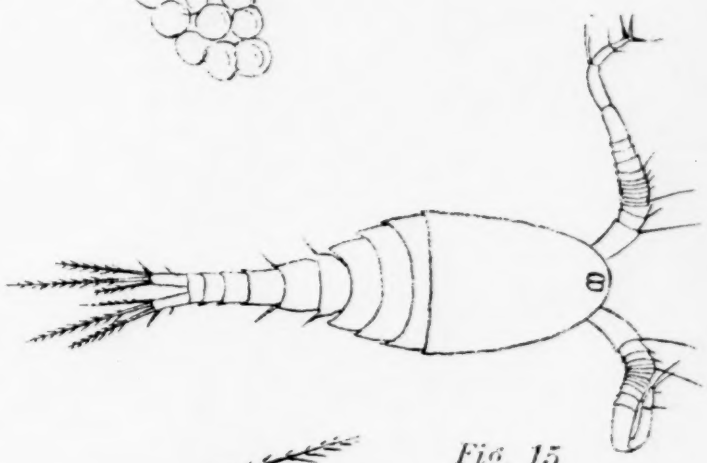

Fig. 15

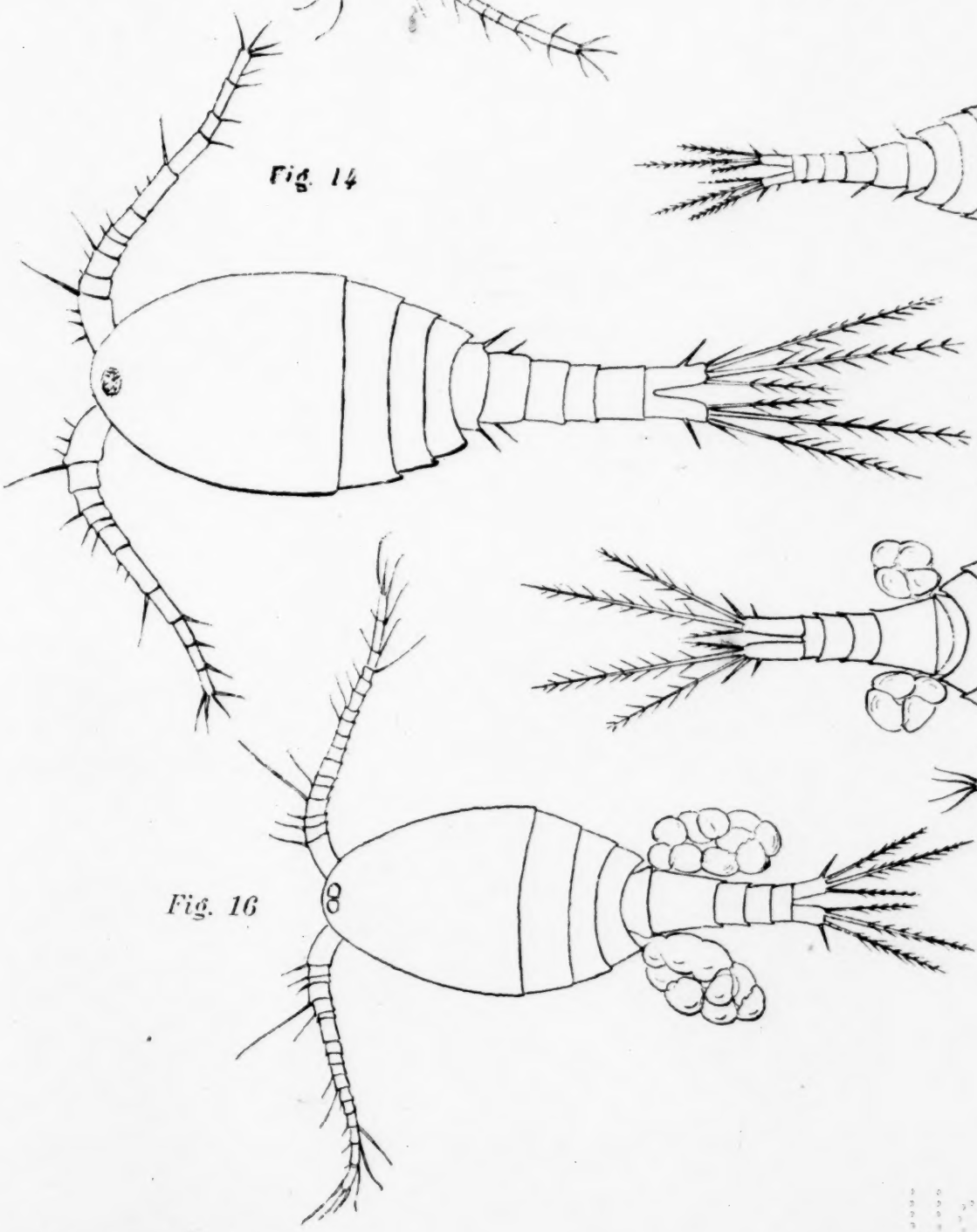

Fig. 14

Fig. 17

Fig. 16

W. M. Graham, ad. nat. del.

EXPLANATION OF PLATE XXXVI

DIAPTOMUS INNOMINATUS, sp. nov., Brady

Fig. 18.—Female. × 124.
Fig. 19.—Male. × 124.
Fig. 20.—Male, fifth pair of legs. × 102.
Fig. 21.—Female, fifth pair of legs. × 102.

ATTHEYELLA AFRICANA, sp. nov., Brady

Fig. 22.—Male. × 74.
Fig. 23.—Female. × 74.

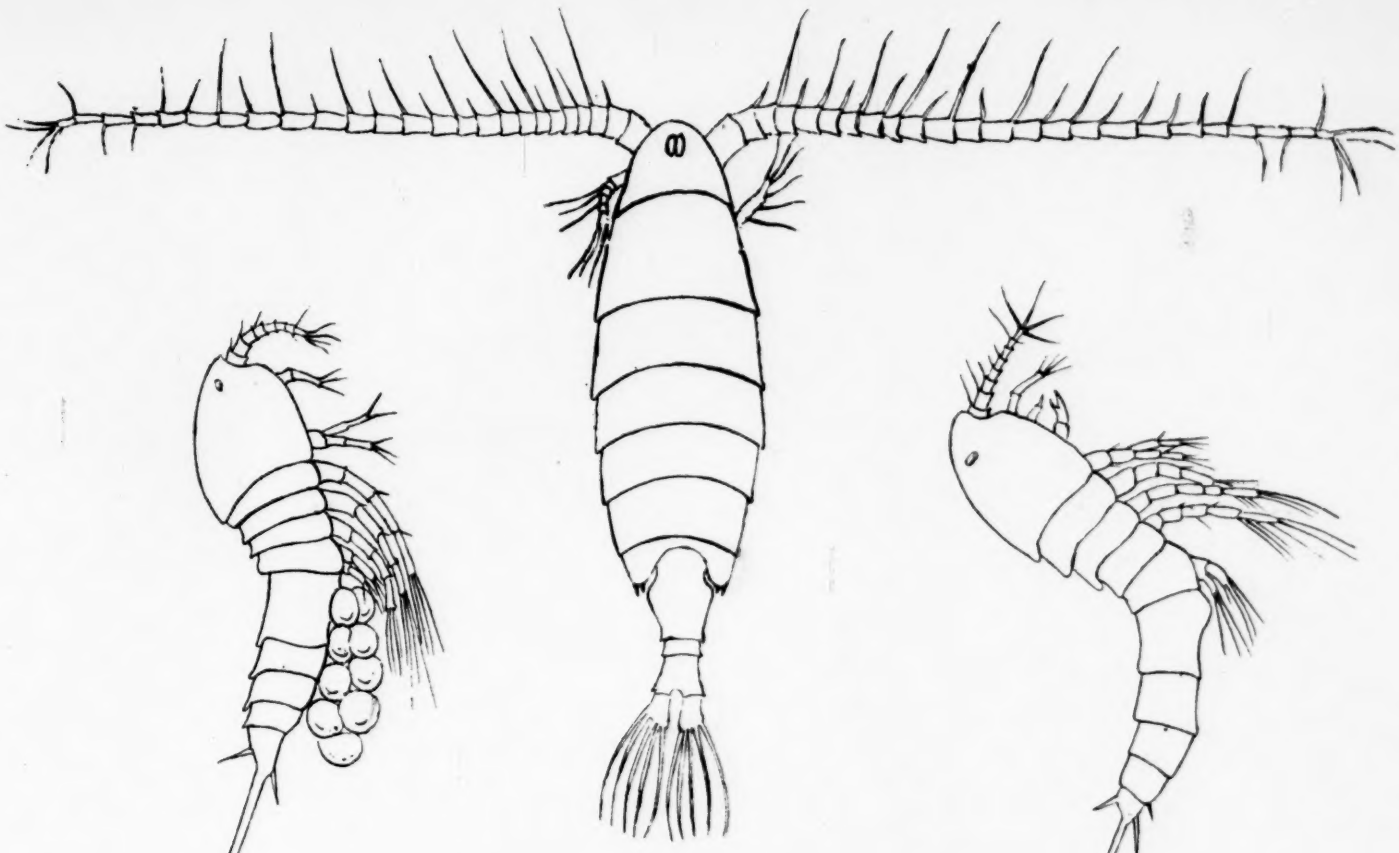

Fig. 18

Fig. 22

Fig. 23

Fig. 19

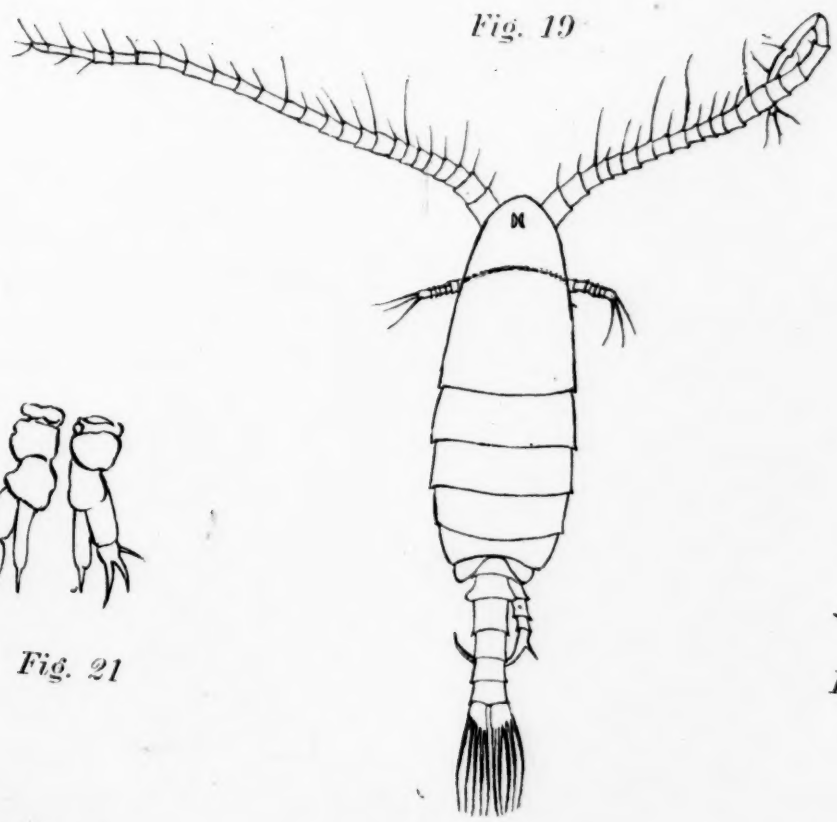

Fig. 21

Fig. 20

W. M. Graham, ad. nat. del.

ON THE MORPHOLOGY AND LIFE HISTORY OF *SPIROCHAETA DUTTONI*

BY

ANTON BREINL, M.U.Dr. Prag

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From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine

(*Received July 1st, 1907*)

In the course of our experimental study of the spirochaete of African Relapsing fever, *Spirochæta duttoni*,[1] numerous films were made of the blood, and of the organs, of animals in the different stages of the disease, with a view of ascertaining the life-history of the parasite. Considerable uncertainty still exists concerning the morphology and the life cycle of the whole group of spirochaetes. While Novy and Knapp[2] deny that the parasites undergo any morphological changes, Prowazek,[3] in his work on fowl spirochaetes, gives them an undulating membrane and a definite nuclear apparatus, and also describes intra-cellular stages. One terminal flagellum was observed by Novy and Knapp in *Spirochæta obermeieri*, and by Stephens[4] in *Spirochæta duttoni*; Zettnow,[5] on the other hand, describes peritrichal flagella in *Spirochæta duttoni*, and Borrel[6] in fowl spirochaetes.

The stain employed in the present work was Giemsa's modification of Romanowsky's stain, in dry films. Wet films were not found to possess any particular advantage when working with this parasite.

The spirochaete consists of a darkly stained central core, which is surrounded by a light-stained periplastic sheath. This sheath extends beyond the central chromatic core, and is drawn out at one end into an elongated filament, thus forming a structure which has been described by various observers as a terminal flagellum. All attempts

to demonstrate peritrichal flagella, either in fresh or in stained specimens, have completely failed. The central core, or chromatic part, does not always stain uniformly, but in certain parasites lighter and darker areas are noticeable, either throughout the whole length or confined to one part of the parasite (figs. 1, 2). Very frequently, especially in spirochaetes which are disappearing from the circulation, the whole chromatic core seems to be broken up into an irregular number of granules (fig. 3). In this stage of infection the spirochaetes often show one or more swellings, either in the centre or at one end (figs. 4, 5). A fairly constant appearance, which has been previously described by various observers in *Spirochæta obermeieri* and *Spirochæta duttoni*, is a small unstained transverse band situated at about one-third of the length of the parasite (fig. 6).

A considerable amount of work was done with the object of demonstrating an undulating membrane. Although in specimens stained with Giemsa's solution an appearance was sometimes seen which resembled an undulating membrane, this was, in my opinion, due only to the flattening out of the spirals of the parasite. In wet films, even after a prolonged staining by Heidenhain's method, no trace of an undulating membrane could be seen.

The division of *Spirochæta duttoni* is, as a rule, transverse. The parasites increase in length and become thinner in the middle; this thinner part then elongates more and more until the two individuals separate (figs. 7, 8). It is very probable that the unstained area frequently seen in the normal parasite (fig. 6) is the point of the future elongation and subsequent transverse division. Occasionally, longitudinal division was seen to take place, especially at the time of the disappearance of the parasites from the peripheral circulation, and in this stage of the infection in the organs the parasite was seen to increase in thickness, the division commencing at one end of the spirochaete and gradually extending along its entire length (fig. 9). Judging from the scantiness of the parasites at this stage, it would appear that this process is a comparatively rapid one. In rare instances, at this stage of the disease, parasites were seen being engulfed by phagocytes.

A striking appearance, as depicted in fig. 10, was, on rare occasions, seen in the blood. Two spirochaetes were observed lying close to each other, touching at certain points. The one was stained

dark red with Giemsa, the other light blue, with apparently no chromatic core, but showing an irregular number of dark red granules situated at the points at which the two spirochaetes were in apposition. We are inclined to explain this appearance as conjugation.

Prowazek describes intra-cellular stages of *Spirochæta gallinarum* in the red blood cells. We were able to observe the same phenomenon in rare instances with *Spirochæta duttoni* just before the crisis set in (fig. 11).

Although the appearance of the parasites in the peripheral blood seemed fairly uniform, striking changes were observed in parasites seen in the organs, notably in the spleen, bone marrow, and liver.

Numerous spirochaetes, especially just before the crisis, when the blood was still swarming with parasites (principally in the spleen and bone marrow, rarely in the liver), were seen coiling themselves up (fig. 12), a few presenting a swollen appearance (fig. 13), the majority gradually becoming thinner and rolling themselves up into more and more complicated skein-like forms (figs. 14, 15) which seemed to become more irregular as the time of the crisis drew near The majority of these forms were devoured by the phagocytes of the spleen, and at the time of the crisis the spleen cells were observed to be gorged with degenerated spirochaetes. In animals in which the spleen had been removed an analogous process took place in the liver.

A few similarly shaped parasites underwent a remarkable change:—The outline remained more regular for a time, and the parasite surrounded itself with a thin cyst wall, the interior of the cyst being filled with a faintly blue stained plasma (fig. 16). These forms, in scanty numbers, were to be seen even after all the other forms had disappeared. They apparently undergo further changes, as the shape of the parasite becomes more and more indistinct and, at a still later stage, only the cyst filled with small red granules persists.

We were unable to trace the further development of these forms, as in specimens stained by Giemsa's method it is impossible to differentiate them from blood platelets and other constituents. The fact that the filtrate of spirochaetal blood through a Berkfeld filter is infective suggests that these small granules may be the forms which pass through the filter and give rise to a fresh infection.

The life history of the spirochaete might be thus summarised:—Just before the crisis the spirochaetes disintegrate, certain of them coiling up into skeins, the majority of which are phagocytosed by the spleen. Some of them become encysted and break up into very small bodies, out of which the new generation of spirochaetes is evolved.

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   Zettnow. Geisseln bei Hühner und Recurrensspirochaeten. "Deutsche Medizinische Wochenschrift," March 8, 1906, page 276.
6. Borrel. Cils et division transversale chez le Spirille de la poule.
   "Comptes rendus de la Société de Biologie, Tome 60, p. 238, 1906.
   Some forms described in the present paper were observed by this author.

EXPLANATION OF PLATE

The accompanying drawings were done with a Zeiss apochromatic objective 2 mm. aperture 1·4, ocular 18. Drawn to the scale of 4,500.

Figs. 1 to 12.—From the peripheral circulation of infected monkeys and rats, respectively.

Fig. 13.—From the liver of an infected monkey.

Figs. 14 to 16.—Forms found in the spleen.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 7

Fig. 5

Fig. 6

Fig. 8

Fig. 9

Fig. 10

Fig. 11

Fig. 13

Fig. 14

Fig. 16

Fig. 12

Fig. 15

PLATE XXXVII

THE
CYTOLOGY OF THE TRYPANOSOMES

THE CYTOLOGY OF THE TRYPANOSOMES

PART I*

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(*Received July 15th, 1907*)

CONTENTS

(A) INTRODUCTORY

The trypanosomes belong to a group of organisms of great practical importance, since they are related to numerous diseases, not only affecting many valuable animals, but, in the case of sleeping sickness, man also. Notwithstanding the facts, the nature and

* A preliminary account of the observations relating to *T. gambiense* contained in the present paper was published in the *Lancet*, p. 1219, May 4, 1907. In a subsequent paper by Plimmer and Thomson received by the Royal Society, July 20, 1907, these authors appear to have encountered either the encysted Trypanosomes, or the resistant bodies (latent bodies) which we had previously described. But from the vagueness of their reference, Pro. Roy. Soc. B. Vol. 79, p. 509, it is impossible to be certain to which order of structures already described by us they do refer.

morphology of these organisms are as yet but little understood. Information upon these matters, as well as upon the various life cycles they appear to present, is greatly to be desired. Especially is this the case with regard to their morphology. The numerous descriptions of their structure and their metamorphoses already in existence have been drawn in general from the results of methods of research not calculated to produce any correct appreciation of their true cytological features. With very few exceptions, the study of the structure of the trypanosomes has been based, either upon what can be made out of the live animals, or else upon observations made upon material after it has been heated and dried, a method which, to say the least of it, may be shown, so far as the finer details of any cell structure are concerned, to be particularly barbarous.

Trypanosomes, like other unicellular organisms, can, however, be fixed in a great variety of ways which are commonly used during cytological research. The chief manipulative difficulty they present is the unreadiness with which they can be made to take any sort of differential stain. Still this difficulty is not insurmountable; and ordinary preparations may be produced which stain as completely as can be desired.

We have used the following fixatives:—Fleming's fluid; sublimate acetic acid; osmic acid vapour; osmic acetic acid vapour; and formalin vapour.

When the animals have been fixed, it is in all cases desirable to use somewhat special precautions in relation to the stain which may have to be employed, the process adopted depending upon the principle of applying a mordant, or mordants, before the actual stains are used. On the whole, we have found that the fixation with Fleming's fluid is unquestionably the best from a morphological point of view, while the staining methods through which we have obtained the sharpest colouration have been, on the one hand, the double safranine orange methylene blue stain invented by Breinl (see Appendix I); on the other, a slight modification of the Heidenhain iron haematoxylin process (see Appendix II).

As we are dealing in this Memoir (and in future publications which will be related to it) with many different specific forms, it is necessary in the first place to consider the cytology of the trypanosomes, as far as is now possible from a general point of view.

This course is unavoidable owing to the confused terminology which has grown up in the literature, and also on account of the present necessity of making clear the meaning we attach to different names. We have further to define our present conception of the nature of several structures which the trypanosomes possess.

When properly fixed, all the animals we have examined present an elongated cell form. No anterior or posterior extremity can, except in the most arbitrary sense, be defined. The exterior of the protoplasm is differentiated into a thin outer layer or ectosarc (periplast). Among the species with which this paper is concerned, the ectosarc is smooth, and does not present any definite ridges or stripes corresponding to the structure often described in the larger trypanosomes, such as those of the frog, and others.

The protoplasmic structure within the ectosarc consists of a very coarse *spongioplasm* (schaumplasma) containing fine staining granules embedded in its substance, the meshes of this spongioplasmic network being filled by a less stainable cytolymph. It is sometimes said that within the ectosarc; and distinct from the deeper portions of the spongioplasm, there exists a layer—the endoplasma. We have, however, not been able to demonstrate the existence of this subdivision.

The permanent cell structures contained within the ectosarc consist of a more or less central area, which, when subjected to Breinl's stain, assumes a purple colour (see figs. 4, 5, 11, 12, 13). We propose to call the whole of this area the *nucleus*. Within the nucleus there is always to be found a clearly-defined body which stains under the same conditions red, and we propose, for reasons which will become more apparent later, to term this body the *intra-nuclear centrosome* (Karyosome, Innenkorper).

It does not appear to be the case, when the animals are not dividing, that the nucleus can be correctly said to be bounded by any definite membrane. In most instances it appears more correct to say, that there is no definite membrane, but rather that there is a very sharp division between the spongioplasmic network and the finer network of the nucleus.

Near the broad end of the animal's body there is usually to be found a granule, or small group of granules, which stain like the intra-nuclear centrosome. These, whatever their numbers at any particular

period, we propose to call the *extra-nuclear centrosomes* (blepharoplasts). From one or more of these granules there springs a staining core, or flagellum, which lies in a thin expansion of the ectosarc, forming the so-called undulating membrane.

For present purposes we have thus the following terminology:—

Ectosarc = (Periplast).

Spongioplasm = (The substance of the network of the protoplasm).

Cytolymph = (The substance between the meshes of the spongioplasm).

Intra-nuclear centrosomes = (Karyosomes, Innenkorper).

Extra-nuclear centrosomes = (Blepharoplasts, micro-nuclei centrosomes, nucleoli).

Flagellum.

Undulating membranes.

In none of the trypanosomes which we have studied have we found the slightest indication of the existence of the so-called males, females, and indifferent forms. We have found that the often-asserted existence of these three types in the blood, a suggestion originating chiefly from Schaudinn,* is totally misleading.

So far as is at present known, trypanosomes are parasites inhabiting the blood, and body fluids, of a great variety of animals. Hitherto no non-parasitic forms have been discovered. As is the case with other parasites of this description, their life histories appear to have become modified to secure their transference from one host to another in different ways. When introduced into the blood and tissues of a suitable host, they usually multiply by fission until either the noxious influence of the infection destroys the host, as in the case of some strains of *T. gambiense* introduced into rats, or the infection runs a different type of course. In the latter type of infection the multiplication of the parasites in the blood rises to a first maximum, and then falls, so that the numbers may decrease to zero. After this fall, which we may speak of as the first negative phase, the parasites reappear, and reach a second maximum, and so on. In such cases, the infection follows an irregular course, which can be easily understood from the diagram given on page 449.

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* For further information respecting this matter see Thomas and Breinl, Liverpool School of Tropical Medicine, Memoir XVI.

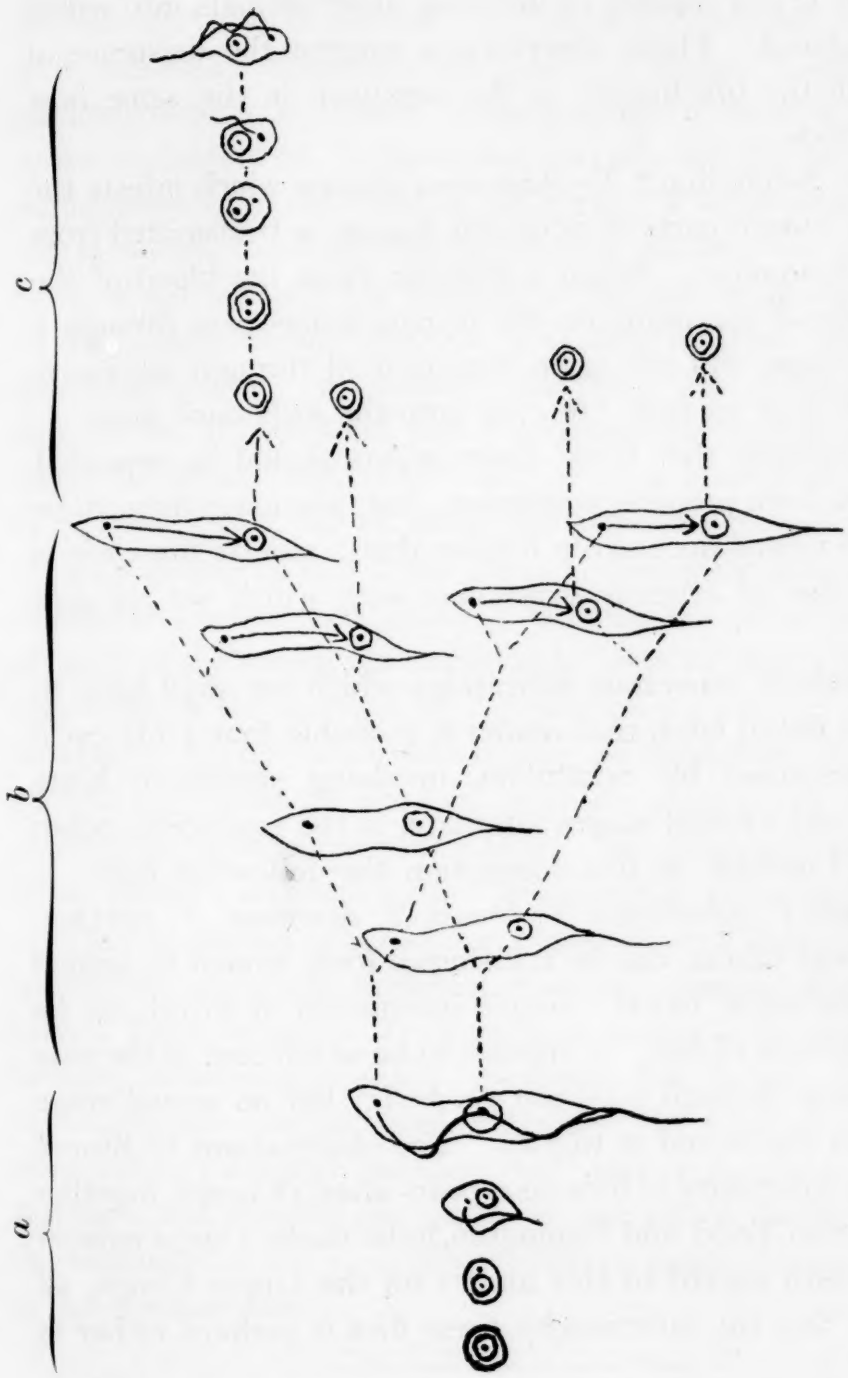

DIAGRAM.—Showing the life cycle of *Trypanosoma gambiense* in a rat:—

a. Development of the Trypanosome from the latent body.

b. The fission of the Trypanosome and the formation of the black line.

c. Reproduction of the latent bodies and the development of the Trypanosome therefrom.

It is a very remarkable fact that in some examples of the latter type of infection the blood during the negative phases, although apparently containing no trypanosomes at all, and even if it be properly filtered, is still capable of infecting other animals into which it may be introduced. These observations suggest the existence of another stage in the life history of the organism in the same host during such periods.

According to Schaudinn,* *Trypanosoma noctuæ* which infests the blood of owls in certain parts of Southern Europe is transmitted from owl to owl by mosquitoes. When withdrawn from the blood of the owl into the body of the mosquito the trypanosomes pass through a definite sexual stage, and are again reintroduced through successive bites, in another stage of their life cycle into the owls once more.

It is very desirable that these observations should be repeated. They have never been properly confirmed; but, assuming them to be correct, it by no means necessarily follows that a similar life cycle is pursued in the case of other trypanosomes with which we are now acquainted.

There are, indeed, numerous indications which we shall have to consider in more detail later, that render it probable that a life cycle such as that described by Schaudinn, involving successive hosts wherein sexual and asexual stages alternate, is the exception rather than the rule. Consider in this connection the following facts:— The trypanosomes *T. gambiense, T. brucei, T. equinum, T. equiperdum, T. lewisi* and others, can be transferred from animal to animal by simple inoculation of blood. Simple inoculation of blood can be effected by many sorts of flies. It appears to be so effected in the case of sleeping sickness through *Glossina palpalis*; but no sexual stage has hitherto been discovered in this fly. The observations of Bruce† showing that the infectivity of flies disappears after 48 hours, together with those of Dutton, Todd and Hanington,‡ who made a large number of experiments with regard to this matter on the Upper Congo, all seem to indicate that the infection by these flies is perhaps rather in

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* Schaudinn. Generations und Wirtswechsel bei Trypanosoma und Spirochaete. Arb. a. d. kaiserl. Gesundheitsamte, Bd. XX, H. 3.

† Bruce, Nabarro & Greig. Reports of the Royal Society. Sleeping Sickness Commission, No. 4, 1903.

‡ Dutton, Todd and Hanington. Trypanosome transmission experiments. Ann. Trop. Med. and Parasit., Vol. I, No. 2, p. 199.

the nature of an accident than a necessary process involved in the normal life cycle of the parasites. Some such conclusion is borne out by other facts in relation to trypanosome infection. Thus in the case of *Dourine*,* simple inoculation of blood will transmit the disease, but it is habitually communicated amongst horses in quite a different manner, namely by coitus. Consequently, if there is a sexual stage in the life history of *Trypanosoma equiperdum*, this sexual stage must occur normally in the body of the horse. Further strains of trypanosomes, such as those of sleeping sickness and *Dourine*, may be kept for years in our laboratories through inoculation from animal to animal. In fact, such strains may be continued in this way for a quite indefinite period, a process involving an endless number of generations in the blood, and it consequently follows that if in such forms the sexual stage occurs only in some other host, this phase can be dispensed with for an altogether indefinite period.

As a matter of fact, there are yet other observations bearing upon Schaudinn's researches, which if they do not necessarily render his account of the sexual act improbable, seem to clearly indicate that it may exist in the instance of *Trypanosoma noctuæ* as a very unusual exception, an exception which may be incapable of throwing any general light upon the life history of the great group of organisms to which *Trypanosoma noctuæ* belongs. We may refer also particularly to the author's account of what he regards as the reduction process.

This, according to Schaudinn,† amounts to a sexual determination, or differentiation, accomplished through a nuclear division. That is to say, there occurs in *Trypanosoma noctuæ* a division (heteropolar mitosis, Schaudinn), which separates the female moiety of an hermaphrodite nucleus from the male. In other words, Schaudinn resuscitates (although he does not appear to allude to this fact) Balfour's and Minot's view of the formation of the polar bodies, and the extrusion of the so-called residual corpuscle during the formation of the spermatozoa.

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* Rabinowitsch and Kempner. Centralblat für Bakt. Bd. XXXVII, H. 5.
See also Minchin, Gray and Tulloch. Pro. Roy. Soc., London. Vol. 78, 1906.
See further Laveran and Mesnil. Trypanosomes et trypanosomiases. Paris, 1904.

† Schaudinn: Neuere Forschungen über die Befruchtung bei Protozoen. Verhandlungen der deutschen zoologischen Gesellschaft auf der XV. Jahresversammlung. Leipzig, 1905.

It is necessary to be quite clear about this matter. The hypothesis respecting the function of the polar bodies, and so-called residual corpuscle of the spermatozoa, as the means by which the opposite "sex-stuff" is got rid of from the ovum, and the spermatozoon, has for various reasons collapsed some years ago. In the first place, the polar bodies cannot be homologised in any way with the residual vesicle. In the second place, it has been clearly demonstrated that in the vast majority of animals and plants the sexual reproductive cells, ova, and spermatozoa are precisely equivalent so far as their nuclei are concerned.* Reduction as now understood in animals and plants is not sex differentiation, but a process which results simply in the halving of the number of chromosomes in those cells which are destined to conjugate.

A great deal of confusion has been produced by Schaudinn's inaccurate use of the term reduction, a term which in general biology has long had a limited and a definite meaning. In dealing with these matters it must, therefore, be clearly understood that by reduction Schaudinn means sex differentiation, and that the term reduction in general biology does not mean sex differentiation, and stands for something quite different.

(B) THE MORPHOLOGY AND LIFE CYCLE OF TRYPANOSOMA GAMBIENSE

Trypanosoma gambiense (Dutton) is a parasite associated with the disease appearing in Equatorial Africa and known as Sleeping Sickness. It can be transferred by simple inoculation into nearly all the animals generally used for laboratory experiments. The infection may run a very varied course. Thus the strain used in Liverpool for inoculation into rats may simply increase until, within a period of a few days, the rat's blood is teeming with parasites and the animal dies from the effect of the invasion. In other cases among the rats, as in man, the number of parasites in the blood rises and falls in a

* It is unnecessary to refer to the vast literature of this subject. The repeatedly confirmed observations upon which the above statements are based, being so well known as to render it unintelligible why Schaudinn did not himself make it clear that the process he describes, under the term reduction, can have nothing in common with the reduction process as described and studied in animals and plants for the last 20 years. It is equally unintelligible why this author did not point out the identity between the process he discussed and that erroneously supposed to exist among animals and plants by Balfour and Minot.

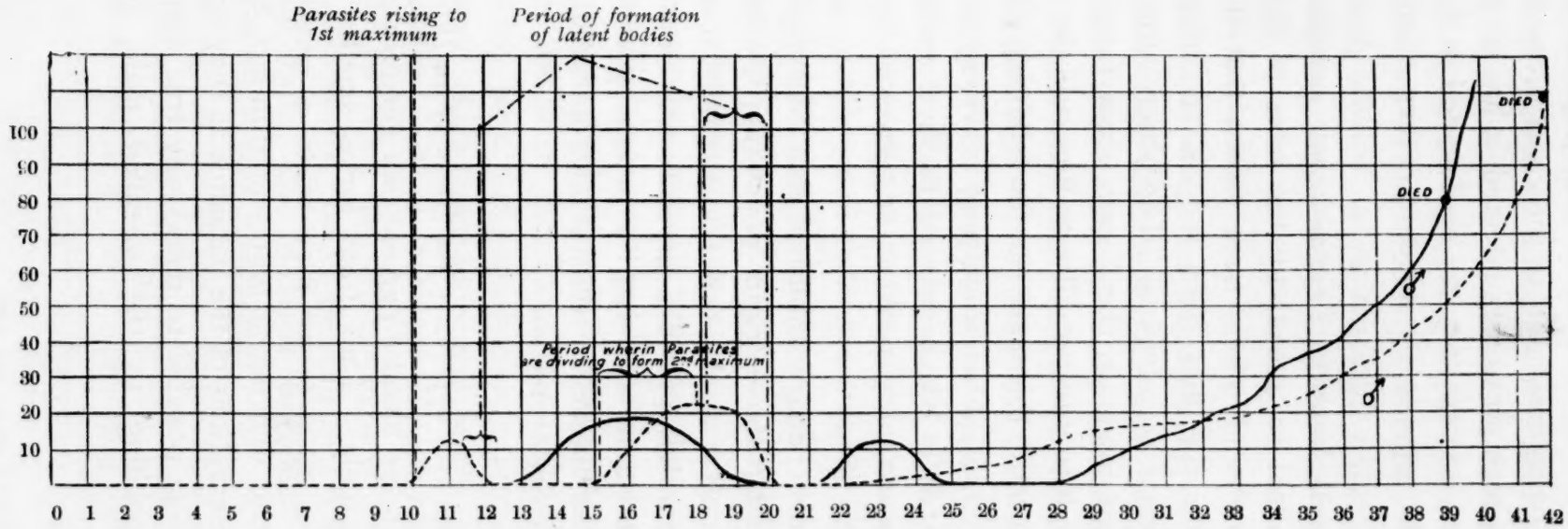

CHART OF TWO MALE RATS INOCULATED WITH *Trypanosoma gambiense*.

The horizontal figures represent the days after inoculation: the vertical figures the numbers of parasites in a microscopic field of blood; the curve representing the variation in this during the course of the infection. The two different curves represent two different infections.

somewhat irregular series of well-marked periods, the kind of oscillation produced being indicated in the chart given on page 449. When an animal has been previously infected, it has been found that even at a period when no parasites can be detected in the blood, the blood is nevertheless capable of infecting other animals by sub-inoculation.

As is already well known, *Trypanosoma gambiense* can be transmitted from animal to animal by the bites of flies; but the observations of Bruce* and others have shown that if more than 48 hours elapse after the flies have fed on an infected animal, subsequent bites produce no infection. The observations of Dutton, Todd and Hanington† made on the Congo, show further that it is often extremely difficult to infect at all with flies, and the authors sum up the position in respect to this matter in the following paragraph:—

"We believe either, (I) That something is wrong in the way in "which *Glossina palpalis* has been used in these experiments, or, "(II) That *Trypanosoma gambiense* can be conveyed by some other "means than by it."

So far, then, from it being established that Sleeping Sickness is normally spread among the African population by the bites of *Glossina palpalis* alone, it would seem that the most recent work on this subject indicates that possibly the infection through flies is in the nature of an accident, and that the means by which Sleeping Sickness spreads, in the manner in which it does spread in the African interior, has yet to be discovered.

Trypanosoma gambiense as it appears in the Blood of an Infected Animal

When examined in the blood *Trypanosoma gambiense* is found to vary in size very largely. Thus from forms smaller than those represented in figs. 1, 2, 4, 6, we may select a series increasing gradually to the extreme dimensions represented in figs. 8, 9, 10.

From our own observations of the parasites as they appear in the blood, it does not seem possible to detect any true dimorphism, or trimorphism, corresponding to the so-called male, female, and

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* Bruce, loc. cit.

† Dutton, Todd and Hanington, loc. cit.

indifferent forms described by Schaudinn,* Minchin,† and many other authors. The present examination of the forms appearing in the blood leads us to believe that there is to be found among these trypanosomes a series extending from those which are relatively small, to those which are relatively immense. The three forms often described and alluded to as distinct, consequently appear to be arbitrarily chosen examples in a continuous series of dimensions.

Multiplication in the Blood

From the time when the parasites appear in the blood of an infected animal until their numbers reach any particular maximum, rapid multiplication takes place by longitudinal fission of the individual trypanosomes, the multiplication being most rapid near the successive periods of maximum numbers. When the parasites are not dividing, they present the appearance represented in figs. 1 and 4.

The nucleus is nearly in the middle of the long axis of the cell, and consists of an outer stainable mass, enclosing generally a lighter central area, within which there lies a small, sharply-definable body, which stains red in contrast to the purple colour of the outer mass when subject to Breinl's stain. This central structure forms the intra-nuclear centrosome (karyosome). At the broad end of the cell there exists another staining granule which, when the cell is not dividing, remains single, the granule in question forming the extra-nuclear centrosome (blepharoplast, micro-nucleus, centrosome). It stains, under the above conditions, like the intra-nuclear centrosome. Arising directly from the extra-nuclear centrosome, there extends a delicate thread, which stains more faintly, but in the same manner as the centrosomes. It is enclosed in a thin expansion of the ectosarc running along the entire length of the cell. The thread projects at the narrow end of the cell as a long stained whip-lash. This thread forms the so-called flagellum, and the ectosarcal expansion the undulating membrane.

The first sign of an approaching fission is generally apparent in relation to the extra-nuclear centrosome. From this there buds out a small fragment, figs. 1, 2, and 3, which may become flattened, as in

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* Loc. cit.

† Pro. Roy. Soc., 1907.

figs. 1 and 7, and can be seen to be attached to the original extra-nuclear centrosome by faint unstaining strands, fig. 7. At a later period there grows out from the new extra-nuclear centrosome a young flagellum, which gradually extends parallel with the old flagellum, as in figs. 2, 3, 6.

At a period of the fission roughly corresponding to the above, it can be observed that the intra-nuclear centrosome has also divided in the manner represented in figs. 2, 3, and that the staining outer portion of the nucleus has gradually collected around, and beyond, the dividing portions of the intra-nuclear centrosome. In this form of nuclear division characteristic of *Trypanosoma gambiense*, no chromosomes are formed at all, and the intra-nuclear centrosome behaves precisely as the so-called karyosome, or centrosome, during the division of the nucleus in *Euglena*, *Eimeria schubergi* (Schaudinn), and other protozoa. At a later stage the division of the intra-nuclear centrosome becomes complete, and the outer staining portion of the nucleus collects round the two intra-nuclear centrosomes as in figs. 3, 7, 8, forming together with these two bodies two daughter nuclei, having just the same appearance as the parent nucleus. For all practical purposes the nuclear division of *Trypanosoma gambiense* is thus amitotic; but it is a form of amitosis somewhat complicated by the presence of an intra-nuclear centrosome.*

In the remainder of the cell, the process of division proceeds by the extension and growth of the new flagellum through stages such as represented in figs. 11, 12, 13, 14, so that two apparently similar flagella are produced, each enclosed in an expansion of the ectosarc. They form separate undulating membranes and stretch from one end of the animal's body to the other, fig. 12. The extremities of the flagella become eventually separate, and the animal gradually splits from the narrow end towards the broad end, figs. 13, 14. The daughter forms finally separating, pass through stages such as those represented in figs. 13, 14. In this way we have eventually two trypanosomes, each exactly resembling the parent form, but smaller.

During the growth and division of the parasites in the blood, it is frequently possible to find large numbers of cells in all stages of

* In connection with this matter it should be noted that in many of the metazoa where the centrosomes are always extra-nuclear, these bodies may be definitely related to amitosis when this form of division occurs.

division, and rest, wherein there exist, especially towards the narrow end of the cell, quantities of granules that stain under the action of Breinl's stain an intense red. The colour of these granules is quite distinct from that of either the intra-nuclear centrosome or the extra-nuclear centrosome, and they cannot be stained with any satisfaction at all by means of the iron haematoxylin method. They are quite irregular in size and number, and also in their appearance, that is to say, they may appear in all the trypanosomes in all their stages at one period, and not at another. From these circumstances, we are inclined to regard these granules as of metabolic origin, and we can find no evidence that they arise from the nucleus at any time.

These bodies should, however, not be confounded with the minute granules always seen, more or less throughout the spongioplasmic network of the entire body. These latter may, and very often do, stain in the same manner as the intra- and extra-nuclear centrosomes, but we have been unable to find any indications as to their origin, or that they any more than the large metabolic granules have any special relationship with the nucleus.

It is possible, indeed probable, that some of these granules may correspond to the vegetative and trophic chromidia observed in *Rhizopods* by Schaudinn,* and by Hertwig† in *Actinosphærium eichhorni*, but owing to the very different methods we have used, we are not in a position to make any definite statement with respect to this matter.

At a late stage of division, such as that represented in figs. 14 and 13, the appearance of the organisms at first sight very much suggests an act of conjugation, but in all such cases that we have examined we have found no indication in relation to the nuclei, or the centrosomes which would suggest a conjugational act.

Along with the regular method of division just described certain modifications are frequently observed, which, although producing the most striking appearance (see figs. 8, 9, 10) are nevertheless apparently always capable of being explained through a disparity in the stage simultaneously reached by different parts of the cell. Thus the nucleus may divide completely and then divide again so as to form

* Schaudinn. Arbeiten aus dem kaiserlichen Gesundheitsamte, Vol. XIX, 1903.
† Hertwig. Archiv. für Protistenkunde, Vol. 1.
See also Mesnil. Chromidies et questions connexes. Bull. Past., Tome III, p. 313.

four nuclei, without, however, the extra-nuclear centrosome having divided more than once. Or the extra-nuclear centrosome may divide and form three or four flagella without the nucleus having divided at all. When these unusual methods are adopted, the gigantic trypanosomes figured on Plate I are frequently produced.

Changes in the Trypanosomes relative to the Stage of Infection

The appearances described in the preceding paragraphs are those which are encountered among the parasites during multiplication after their first appearance in the blood of the infected animal. If, however, other stages of the infection be studied there are to be found different morphological appearances among the parasites, which are apparently of the greatest importance. In the case of an animal which has become infected with *Trypanosoma gambiense*, and shows a well-marked oscillation in the course of the disease, it is found that as the number of the parasites is rising in the blood—that is to say, along the ascending portions of the curve in the diagram (page 449)—the phenomena presented are the same as those found in the initial stages of the infection which we have just described. If, however, we study preparations made at or near the maxima of the curve, other changes are found to be taking place in the trypanosomes besides those of simple division.

At the time the curve approaches any maximum, there are to be found trypanosomes which present the appearance shown in figs. 15 to 20. From this figure it will be seen that such trypanosomes are distinct from those which have been previously described, in that a relatively-thick stainable band proceeds from the extra-nuclear centrosome. This thick band is found to be most readily stainable by iron haematoxylin; it is less readily, but still stainable by the various aniline colours which we have employed. It grows from the extra-nuclear centrosome not along the surface of the animal as in the case of the new flagella, but down the interior of the cell towards the nucleus (see figs. 15, 16).* This stainable band, which appears near

* It is probable that the band we here refer to is the same as the structures described by Prowazek, Studien uber Saugethier trypanosomen. Arb. a. d. kaiserl. Gesundheitsamte, Band XXII, 1905, and also Miss Robinson, Notes on certain blood-inhabiting protozoa. Proceedings of the Royal Society, Ed. Vol. XVI, No. 6.

the periods of the greatest number of parasites in the blood, is fully twice as thick as the flagellum. As it increases in length it may reach, or even pass the nucleus; or it may become coiled upon itself, as in figs. 16, 17, 18. But whatever form it takes at first, the later appearance suggests that the band eventually becomes in one way or another definitely connected up with the nucleus. This suggestion is strengthened by the subsequent behaviour of the band, for it is seen eventually to become gradually less stainable, to break up into a string of fragments, and finally to disappear. Through all these later stages of its existence it is most certainly suggested, as in figs. 19, 20, that it is directly related to the nucleus, that is to say, to have been, or to be actually connected up with it.

We appear then, at or near the maximum number of the parasites in the blood, to have direct evidence of some sort of interaction taking place between the extra-nuclear centrosome and the nucleus. This phenomenon occurs only among animals in which no evidence of nuclear division, or cell division, is apparent. In such cases, we do not find that either the extra-nuclear centrosome or the nucleus is divided, and throughout the whole development of the stainable band the nucleus remains in a condition of complete repose.

If now we examine the portions of the curve of infection where the trypanosomes are decreasing in number, still other phenomena make their appearance. The numbers of the parasites gradually decrease in the peripheral blood, and at this time, in such organs as the lungs, the spleen, and the bone marrow, we find curious changes to be taking place in vast numbers of the trypanosomes encountered in these situations. Parasites showing the present changes are rarely found in the organs named above during the period when the number of the parasites is increasing in the blood, but at the time when the numbers are beginning to decrease we see in the lungs that numbers of trypanosomes show alterations in their nuclei. The protoplasm of the animal's body becomes detached from the periphery of the nucleus which lies in a clear space, while the nucleus itself contracts somewhat, and at the same time a large clear vesicle gradually grows up in connection with it, in the manner represented in figs. 22, 23. Round the outside of this vesicle and the nucleus there may be seen a layer of protoplasm enclosing in a delicate sheath both nucleus and vesicle together. When this stage has been reached, the rest of the cell body

rapidly disintegrates, the outline of the cell becomes lost, and the flagellum together with the extra-nuclear centrosome may be frequently seen detached, and lying loose among the cells of the organs examined (see fig. 26).

These phenomena, as we have said, are encountered in the lungs, but they are also found in the bone marrow and the spleen. After the above stage has been reached the nuclear bodies and the disintegrating remains of the parent cells disappear to a large extent from the lungs, the nuclear bodies being subsequently found in very large numbers both in the spleen and the bone marrow.

At first sight, the appearance we have just described might be supposed to be simply a phase in the disintegration of the parasites, and this was, as a matter of fact, the view which we were at first inclined to adopt. There are, however, reasons for thinking that, although the greater part of the protoplasm forming the bodies of the parasites undoubtedly does disappear, the peculiar nuclear structures we have just been considering do not follow the same course. After their detachment from the rest of the disintegrating cell body, the nuclear corpuscles become impacted in great numbers in the spleen and bone marrow. Here, instead of disappearing in the course of a few hours, they may remain intact for as much as ten days or more, in fact, throughout the whole of the negative or latent period of the infection, when no parasites are present in the peripheral blood. From the time of their formation, these peculiar bodies,* which we propose to term *latent bodies*, may be found in small numbers in the blood, and although they thus remain intact for a relatively long period, it

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* The formation of these bodies appears not to have been previously observed at all in *Trypanosoma gambiense*; but similar bodies have been seen in relation to other trypanosomes, and have been variously interpreted. Thus, Rodet and Vallet, Arch. des Med. expériment., Vol. 18, No. 4, describe bodies which appear to be similar to the latent bodies of *Trypanosoma gambiense* in *Trypanosoma brucei* in the blood of the organs and regard them as degenerate forms. Plimmer and Bradford (Quar. Journ. Micro. Sci., Vol. 45) seem also to have found them in the spleen, &c., during *Nagana* infections, and describe them as apparently the nuclei of a plasmodial production. Laveran and Mesnil describe apparently similar bodies as "formes d'involution." Lingard (Journ. Trop. Vet. Sci., Vol. 2, No. 1) appears to have seen them in the blood of cattle infected with *Trypanosoma indicinum*, and to regard them as forming part of the developmental cycle. Holmes (Journal of Comparative Anatomy and Therapy, Vol. 17, 1904) figures in detail the formation of precisely similar bodies in the case of *Trypanosoma evansi*, and regards these bodies as true portions of the life cycle, the illustration of the details of the formation of the latent bodies in this paper being extremely accurate. The latent bodies we have dealt with in *Trypanosoma gambiense* probably also correspond to the bodies figured by Prowazek in the rat louse.

was still possible that they might eventually simply disappear altogether, the nuclear constituents of the trypanosome body being perhaps more resistant than the rest of the protoplasmic structure of the cell.

In order to throw some light upon this matter, we examined a number of infected animals which had been treated with atoxyl, this substance having the effect of destroying the parasites in the blood in the course of a very few hours at the most. When the blood of such animals was treated by injection of atoxyl and examined during the time when the parasites were still increasing in number, it was found that a large percentage of the trypanosomes could be observed dead among the corpuscles of the blood, but during the rapid disintegration which follows nothing comparable to the formation of the latent bodies is encountered. On the contrary, the nuclei in these instances are among the first of the organs to be affected by a general disintegration, which rapidly produces masses of débris, wherein it is only just possible to recognise from their shapes the remains of trypanosome cells (see fig. 41).

The same appearance is produced through the disintegration and death of the trypanosomes in the blood of an animal which has been killed by the disease.

From all this, it would appear that the latent bodies are not produced during the ordinary course of cell disintegration, and must be considered from some other point of view.

At this point it is, however, necessary to explain that when animals are injected with atoxyl at a time when the trypanosomes are decreasing in numbers in the blood, the disintegration does not necessarily overtake all the trypanosomes present. It is found, in fact, that a certain number of trypanosomes under such conditions do not succumb to the effects of the drug, but round themselves up and become encysted (see figs. 37, 40). These cysts are, however, very much larger than the latent bodies. They appear to be true resistant forms produced directly in response to the drug, and are not in any way comparable to the latent bodies we have just described.

With regard to the latent bodies which are produced at the maxima of an oscillating infection (structures which at first sight might, and probably would, be taken for disintegration products), these are, as we have seen, eventually collected in the spleen and

the bone marrow, and do not necessarily degenerate there. They persist in such situations in very large numbers, and each consists of a flattened nucleus with an intra-nuclear centrosome. There is also a vesicle attached to the nucleus, and the whole nuclear apparatus is surmounted by a thin film of protoplasm, figs. 27, 28.

At the periods when there are no trypanosomes to be found in the blood, these peculiar latent bodies are all the evidence of the existence of the parasites in an infected animal to be detected microscopically.

At the period of the infection when a few parasites begin to reappear in the blood, it is possible to still find numbers of latent bodies in the spleen, and in the bone marrow, wherein the intra-nuclear centrosome has divided into two, fig. 28.

Again, at this period it is possible to find forms in which one-half of the dividing intra-nuclear centrosome has passed out of the nucleus, fig. 28, forming an extra-nuclear centrosome. Still further, at a later period, we find forms in which a short flagellum has grown from the extra-nuclear centrosome, and these bodies subsequently appear to gradually transform themselves into small trypanosomes in the manner represented in figs. 28 to 32.

As the latent bodies are gradually transformed into small trypanosomes, the number of these bodies in the spleen and bone marrow diminishes, but it appears to be the case that a proportion of what are apparently latent bodies never really develop into trypanosomes and disappear altogether, or, in other words, that only a proportion of these bodies are under the circumstances above described capable of surviving the negative period, and once more forming themselves into complete trypanosomes.

The changes which we have now described are all those which we have hitherto been able to detect in relation to the succeeding stages of the infection in rats.

At first it was anticipated that further changes might have been encountered during different periods of the day and night, but although we have had the parasites in various animals under observation continuously at all periods of the curve of infection throughout several days and nights, no regular nocturnal alteration was discernible. However, it was found that the rapid diminution of the number of parasites almost invariably took place between 2 and 5 a.m.

So far as the above observations upon the life cycle of the

parasite of sleeping sickness have been carried, they appear thus to indicate a complete cycle in the blood of a single host, and the stages of such a life cycle can be semidiagrammatically represented in the manner given on page 445.

(C) THE MORPHOLOGY AND THE MULTIPLICATION IN THE BLOOD OF TRYPANOSOMA BRUCEI

The appearance of *Trypanosoma brucei* in the blood is represented in figs. 42, 43, 44, 45, 46. The chief morphological distinctions which the parasite of the disease Nagana presents when compared with *Trypanosoma gambiense* are found in relation to the distribution of the nuclear substance, and the characters of the extra-nuclear centrosomes.

The division of this trypanosome in the blood is longitudinal, as in the case of *Trypanosoma gambiense.* The nucleus divides amitotically. The division being first marked by a lengthening of the extra-nuclear centrosome until this body finally separates, and to two minute beads. At the same time the nuclear substance also elongates until we observe forms such as those represented in figs. 43, 44, 45, 46.

As in *Trypanosoma gambiense*, the stages of the division of the extra-nuclear centrosome and that of the nucleus may not be the same, at any particular time, and through this circumstance we observe the same sort of multiple, and abnormal forms as in the case of *Trypanosoma gambiense.*

(D) THE MORPHOLOGY AND THE MULTIPLICATION IN THE BLOOD OF TRYPANOSOMA EQUINUM

In the blood, *Trypanosoma equinum* possesses much the same shape as that of either *Trypanosoma gambiense* or *Trypanosoma brucei*; the nucleus is, however, usually placed nearer to the broad end of the cell. The extra-nuclear centrosome is large, and the nuclear division which takes place during the fission of the cells possesses points of much interest, since the centrosomes are more conspicuous in *Trypanosoma equinum* than in many other forms we have examined. The changes which take place in the intra-nuclear centrosome during the division of the nucleus can be studied with great clearness.

When the nucleus is at rest the intra-nuclear centrosome is surrounded by a light space, which is in turn enclosed by the stainable nuclear substance (fig. 47). During division the intra-nuclear centrosome divides, as in figs. 48 to 52. The nuclear substance becoming at the same time collected up in the region of the dividing intra-nuclear beads. As this process continues, the nuclear substance eventually forms itself into two cup-shaped masses situated around, and beyond the intra-nuclear centrosomes. Owing to this, when *Trypanosoma equinum* is dried the edges of these cups becoming flattened down produce at least two irregular bands on each side of the intra-nuclear centrosomes, which under these circumstances may suggest the existence of nuclear chromosomes. This appearance is, however, misleading. The nuclear division of *Trypanosoma equinum* being, as can be seen from figs. 47 to 53, really amitotic, as in the case of *Trypanosoma gambiense* and *Trypanosoma brucei*.

(E) CONSIDERATION OF THE FOREGOING OBSERVATIONS

For the sake of convenience, it is desirable to consider the latent forms as the starting point in the life cycle of *Trypanosoma gambiense*. These bodies, which in an ordinary fluctuating infection may remain unchanged for long periods in the organs (and, to a less extent, in the blood), consist at first of a nucleus containing an intra-cellular centrosome. The nucleus is related to a vesicle, and the whole nuclear apparatus is surrounded by a delicate film of protoplasm. At a later stage, the intra-nuclear centrosome divides into two, and one of these bodies passes out of the nucleus into the outer layer of protoplasm, which gradually increases in extent.

The above process results in the formation of an extra-nuclear centrosome. The extra-nuclear centrosome, and nucleus together with the intra-nuclear centrosome, henceforth form two entirely distinct sets of structures, which remain distinct through a very long series of divisions, as represented in the diagram on page 445, under the letter B.

After the first separation of the extra-nuclear centrosome from nuclear apparatus, both these sets of structures multiply independently throughout the succeeding series of generations until the

period at which the black line is formed. At this period the extra-nuclear centrosome develops a bridge, as it were, and connects itself for the time with the nucleus. It may be assumed that during this period some substance from the extra-nuclear centrosome passes into the nucleus. Anyhow, after this has taken place the remains of the extra-nuclear centrosome are very shortly cast away, together with the greater part of the protoplasm forming the rest of the cell, and the old flagellum.

Thus, if we consider the nuclear apparatus in the latent body as a whole, this would seem to be divided into two parts during the development of the trypanosome. After the formation of the cell is complete, these two structures, the nucleus and the intra-nuclear centrosome, remain in the same state, and multiply independently into similarly distinct bodies contained in the cells produced by all the longitudinal fissions. In other words, there arises from a nucleus A, two new structures, B and C, both of which differ from A. B and C multiply independently as the animals divide, but at a subsequent stage a portion of each B unites again with the C in all the cells, and the condition of the organism immediately reverts to A once more.

We have thus, after the formation of the latent bodies, an unequal division of the nuclear apparatus of the latent body, so as to form two different sets of structures, the nucleus with one centrosome, and the other centrosome by itself. Each of these then multiplies indefinitely in number. In individual cells these structures subsequently unite temporarily, and later the nucleus characteristic of the latent body is produced once again. In other words, dissimilar structures are formed from a nucleus by division, both derivatives multiply by division, and after a time unite in pairs, and the first type of structure is again produced. There is in this process, when contemplated from the present standpoint, an obvious similarity to the two forms of sexual elements in the higher animals and plants; to two sorts of gametic nuclei, or to a sexual dimorphism. A dimorphism in the trypanosomes which is in like manner followed by a reunion, or conjugation between the dissimilar elements, and succeeded by a reversion to the conditions obtaining before the dimorphism was produced.

The procedure in the case of the trypanosome nuclear apparatus differs, however, from that of apparently all other known organisms

where the phenomena of sex are discernible, in that the dimorphic products into which the nuclear apparatus of the latent forms separate remain contained within the same animal during its successive fissiparous generation. With the exception of this difference, however, the phenomena observed are certainly comparable to the production of sexual gametes and their conjugation. In the forms with which we have been hitherto familiar, the retention of the nuclear apparatus related to both sexes in one cell, may thus be nothing more or less than a morphological curiosity, and in no way necessarily suggests that the process in the trypanosomes we have been considering is fundamentally different from an ordinary sexual differentiation.*

Assuming the phenomena with which we have just been concerned to be of the nature of a sexual process, still another view could be held with regard to them. It may be suggested that the metamorphoses connected with the appearance of the black line is an attempt on the part of the trypanosomes to become sexually differentiated, but this attempt is not completed, the cells reverting to their primary condition, in which case the process could be regarded as an example of a special form of parthenogenesis. In relation to the above suggestion, it should be noted that, at the time of the formation of the black line, the whole of the extra-nuclear centrosome does not reunite with the nucleus, but that only one portion of the extra-nuclear centrosome does so. One moiety of the extra-nuclear centrosome is detached from the other, and one of these portions with the flagellum disappears together with the cytoplasm forming the trypanosome body.

We thus start with the separation of an extra-nuclear centrosome from an intra-nuclear centrosome through the division of an original intra-nuclear centrosome in the latent body. But at the end of the cycle, the nucleus enters again into connection with the extra-nuclear centrosome, yet it only does so with regard to a part of this body. On the other hand, it may be that the extra-nuclear centrosome

*It should be noted that there is no nuclear reduction in this process. The intra-nuclear centrosome divides, and one half of this body passes out of the nucleus. Both nucleus and extra-nuclear centrosome then divide in the production of the succeeding fissions. Afterwards the extra-nuclear centrosome or a part of it re-unites with the nucleus, and the rest of the cell body disappears. It is known that during fertilization a centrosome is often brought in with the male element: to this extent the process of fertilization is similar to the above.

divides normally, and that it is the half so produced which enters into connection with the nucleus during the formation of the black line. In this case, it may be that the nucleus receives once more a morphologically complete extra-nuclear centrosome produced in the ordinary way by division. Our observations are not conclusive with regard to these matters.

Considering the whole question from another point of view, it should be remembered that it is only among a percentage of the trypanosomes present during the periods of the maximum numbers of the parasites in the blood which can be found at any time to exhibit the formation of the black line, and we have to assume either that all the trypanosomes which ultimately form the latent bodies have passed through this metamorphosis, or that only some of them have done so.

We have no conclusive evidence upon this question. If, however, it should eventually be shown that only a fraction of the latent bodies are produced from trypanosomes which have passed through the black line metamorphosis, then it will become clear that there are two series of latent bodies, one class produced after the black line formation, and the other without this taking place. Should this be the case, the suggestion that the black line metamorphosis represents a peculiar form of the sexual act, or conjugation, which we have merely provisionally considered, will be found probably to be inaccurate. There will be an actual dimorphism among the latent bodies, and it will in this case be strongly suggested that the actual sexual act has yet to be discovered, and has been overlooked. We think it would be unprofitable to pursue this question further in the present Memoir. It is obviously a question that can only be properly considered after the phases of the life cycles of other trypanosomes are available.

In the introductory portion of this paper it was pointed out that there exists a complete discrepancy between "reduction" as apparently understood by Schaudinn,* and reduction as understood by biologists in general. Without throwing any reflection whatever upon the correctness of Schaudinn's observations in relation to the phenomena exhibited in the life cycle of *Trypanosoma noctuæ*, it is clear that, whether the process he describes exists or not, this process

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* Loc. cit.

has nothing to do with chromosome reduction as ordinarily understood. His conception is a resurrection of Balfour and Minot's idea regarding the function of the polar bodies in the egg, and supposed corresponding structures in the male cells, as machinery whereby the physical representative of the opposite sex is got rid of more or less completely before fertilization.

In our observations upon *Trypanosoma gambiense* and other forms, in which tne life cycle is possibly a complete cycle within the body of one host (see diagram, page 449), we have encountered nothing at all resembling the process stated to take place in the life cycle of *Trypanosoma noctuæ*. Prowazek,* dealing with the morphology and life cycle of *Trypanosoma lewisi*, maintains that the sexual act (in the form of ordinary conjugation) may take place either in the body of the rat-louse, or in the blood of an infected animal—the latter more rarely. According to this author, conjugation is preceded by a reduction process, which he describes in the following words:—

"Wie bereits erwähnt wurde, findet auf den mittleren Stadien "der Verdauung in Mitteldarm die Reduktion der Flagellaten statt, "die aber nicht gleichzeitig die beiden Kerne, den Blepharoplast und "den centralen Kern, erfasst; bald ist der letztere schon völlig "reduziert, während der erstere erst in den Prophasen dieser "Vorgänge steht und umgekehrt.

"Im centralen Kern wird vor der Reduktion zunächst das "Karyosom bedeutend deutlicher und intensiver färbbar, das "Chromatin wird körnig doch vereinigen sich bald diese Körner zu "einzelnen Strängen (Taf. II, fig. 23, 24), die schliesslich nach Art "von vier Reifen das inzwischen geteilte Karyosom umgeben. Dieses "Stadium möchte ich mit den Stadien der Chromosomen Paarung vor "der ersten Teilung der Metazoenspermatogenese vergleichen (Taf. II., "fig. 24). Später findet man in Kernhohlraum wiederum acht mehr "zerstreut liegende Chromosomen (Taf. II, fig. 26). Ein Stadium der "Vierergruppenbildung ist nicht sehr deutlich ausgebildet obgleich "Andeutungen in diesem Sinne vorhanden waren (Taf. II, fig. 25); "doch kann man wegen der Kleinheit des Objektes nichts sicheres "diesbezüglich aussagen, obzwar in dem abgebildeten Fall doch

* Prowazek, loc. cit., page 372.

" 16 Chromosomen, die durch die zwei Teilungen auf vier reduziert
" werden, gezählt werden könnten. Deutlicher waren die Bilder bei
" *Herpetomonas*. Durch die endlich effektiv gewordene Teilung des
" Karyosoms wird der erste Reduktionskorper gebildet, der selten als
" ein dunkles, körniges Gebilde gegen das spitze Ende der Zelle
" abdrückt, sondern meistens dicht am Kern selbst liegen bleibt
" (Taf. II, fig. 30). Bald darauf vollzieht sich noch eine Teilung,
" durch die der zweite Reduktionskorper gebildet wird. In fig. 31 der
" Taf. II, bemerkt man terminalwärts diesen Reduktionskern, der ein
" kleines Karyosom und die vier dicht verbackenen Chromosomen
" enthält. Demnach muss der reduzierte Kern nur vier Chromosomen
" besitzen. An dem sich reduzierenden Blepharoplast kann man
" nicht so viel Details erkennen ; zunächst teilt sich der Blepharoplast
" in zwei Teile (Taf. II, fig. 29), von denen der eine Teil durch eine
" heteropole Spindel noch einer Reduktionsteilung unterliegt. Der
" este Reduktionskorper übernimmt manchmal die undulierende
" Saumgeissel und degeneriert erst ziemlich spät. In anderen Fallen
" bleibt die Geissel an dem reduzierten Blepharoplast haften (Taf. II,
" fig. 28, 29)."

Prowazek describes the nuclear division taking place during the fission of the parasites in the blood in infections with *T. brucei* and *T. lewisi* as being mitotic, and the nuclei as containing eight chromosomes. Our observations upon *T. brucei* and the other trypanosomes with which we have been working, are all similar in regard to this matter. When these animals have been properly preserved there appear to be no chromosomes produced, and the type of nuclear division during the fission of the animals is invariably amitotic, the extra nuclear centrosome and the nuclear substance dividing like drops. It is possible, of course, as in the case of the Ciliata, that the divisions become characteristically mitotic immediately before reproduction, but in none of the trypanosomes which we have examined (one of which, *T. equiperdum*, certainly runs its life cycle in a single host) have we encountered anything of the kind.

We have had these forms under continuous observation for more than a year, and for long periods at all hours of the day and night In the case of *Trypanosoma equinum*, we were at first inclined tc think that the division was of the mitotic type, but this inference was soon found to be simply due to the defective manner in which the

animals had been preserved and dried, or to other forms of indifferent fixation. In other words, there are often produced during fission of the animals under such circumstances appearances in their nuclei due to coagulation effects which may readily be mistaken for nuclear chromosomes. As the fixation becomes better, in all the forms with which we have hitherto dealt, such appearances can, however, be clearly shown to be illusive, and the division of the nucleus during fission to be invariably amitotic in character. The same inference is borne out by a study of the living animals.

Among the illustrations given by Minchin [Proceedings of the Royal Society, Vol. 78, Series B, No. 20, Plate 12], figs. 4, 8, 9, and 17 have been produced from specimens that have been dried and stained, and suggest the existence of chromosomes, but we are inclined to think that these appearances are simply due to the bad fixation methods employed, and are really quite misleading.

With regard to the nature of the nuclear division accompanying fission of the above-mentioned trypanosomes, our results are in complete accord with those of Laveran. Indeed, with regard to the nuclear reduction described by Schaudinn,* and finally by Prowazek, our observations have revealed nothing suggesting anything even analogous to these descriptions. Prowazek gives a series of figures illustrating this process in *Trypanosoma lewisi* (Pl. II, figs. 23, 24, 25). Here nuclei with eight chromosomes are said to be apparent. We cannot detect in the figures themselves the slightest suggestion of this being the case, and are inclined to think that the irregular blotches and strands, undoubtedly correctly drawn, have nothing to do with chromosomes, but are due to the manner in which the specimens have been preserved. In figs. 27, 28, 29, 30 and 31, the so-called reduction of the nucleus as well as the extra-nuclear centrosome (blepharoplast) is represented; but in none of these figures do we see any indication of either true chromosomes, true mitotic division, or true reduction as ordinarily understood. In fact, as far as the illustrations are concerned, we are unable to find, or to see, any indication of a reduction process.

It will thus be observed that the results we have obtained, especially in relation to *Trypanosoma gambiense*, but also equally

* Generations und Wirtswechsel, &c.

† Loc. cit.

among other forms to be described later, differ not only in degree but also in kind from those obtained on the one hand by Schaudinn, and on the other by Prowazek. The descriptions of Schaudinn, in so far as they bear at all upon the present work, do so, however, through the investigations of *Trypanosoma noctuæ*, which since it appears to be a form of trypanosome requiring more than one host for the completion of its life cycle, may very likely differ in the features of this life cycle from the more ordinary forms with which we have been concerned. On the other hand, Prowazek's results obtained in the case of *Nagana* (that is to say, from one of the trypanosomes considered in the present paper), so far as the nuclear changes during fission are concerned, differ entirely from our own; these latter fall directly into line with the observations we have made upon other forms, and are quite incompatible with the description of this process given by Prowazek in the case of *T. lewisi* or *T. brucei*. The question which now confronts us is upon what cause this difference of results depends. We are inclined to think that the difference of result is due to the methods which have been employed. We may as well say here, that from what we have gathered with respect to the different methods that have been generally in use, it appears that all the methods involving the drying of the blood before staining, or, in fact, any method involving drying at all, is, so far as nuclei are concerned, absolutely useless from a cytological point of view. Nothing relating to the delicate mechanism of mitotic division is generally preserved in cells, whether they belong to unicellular or multicellular organisms, when dried and stained with Romanowsky, Giemsa, or in any other manner. Even the resting nucleus itself under such conditions becomes a mere caricature of the actual structure.

When treated in this way, the irregular or regular blotches and streaks of stainable matter have nothing in common with, and do not represent, even in a relative or equivalent sense, the structures actually present in the cells. Anyone who wishes to verify this fact for himself will have no trouble in doing so if, for example, he makes a smear preparation from the testis of a rat, stains after the manner of Romanowsky, and then compares this with a properly fixed and stained smear, in the production of which ordinary cytological precautions have been observed. It is a curious fact that in a rat's testes under these conditions certain cells which really contain

sixteen *gemini* or heterotype chromosomes, when subject to the action of drying and Romanowsky, very often present (within the ill-formed area representing the nucleus) six irregular masses of stainable stuff resembling the so-called chromosomes of the dried trypanosomes. Such appearances are, however, certainly due to regularity of coagulation and shrinking during the drying of the cells, and have nothing in common with the real morphological structures, (the chromosomes), which either the living cells, or successfully-preserved cells possess. In view of these circumstances, we are inclined to regard many accounts of the existence of chromosomes, spindles, and even the assumed existence of mitotic division among trypanosomes, as conclusions which appear to be most questionable, and as requiring in all cases confirmation in a variety of ways which do not involve the violence dealt to the finer details of cell structure by drying.

Finally, we see in the case of *Trypanosoma gambiense* that the life history of this parasite as it lives in rats seems to be complete in the blood of the rat, and not in any way dependent for its completion upon the transference of the parasites into the blood of any other kind of host. In rats the latent forms pass gradually into trypanosomes, these in turn divide through many generations, and their multiplication is followed by a metamorphosis which, whether we regard it as a special form of sexual process, as a form of pathogenesis, or as a sexual stage, the fuller details of which have not yet been elucidated, seems undoubtedly to stand in one of these relationships to the normal cell multiplications preceding the formation of latent bodies. The stage in question results in the production of the latent bodies once more, and the cycle is complete.

It may be objected to this conception that, notwithstanding the cyclic development of *Trypanosoma gambiense*, still there may exist a possibility, or probability, of the transference of the trypanosomes into some other host where a further metamorphosis representing the sexual stage of the organisms is passed through. This, of course, may be so, but we have in the case of the trypanosome of *Dourine* a clear instance of a trypanosome life history, which, under normal circumstances, is not transferred into any other kind of host; and, under normal circumstances, *Trypanosoma equiperdum* must pass through whatever sexual stage it may possess, its whole life history in fact, in

the body of the horse. *Dourine* can, however, like sleeping sickness, be inoculated from host to host by simple transmission of blood as well as by coitus; in other words, the faculty of being transmitted by simple inoculation of blood is shared by *Trypanosoma equiperdum*, wherein no other host is usually involved, as well as by *Trypanosoma gambiense*. In these circumstances, it is simply natural, assuming flies to be the agents by which sleeping sickness is transmitted, to admit that this form of transmission may be merely in the nature of a mechanical transference, and have no more relation to the sexual act in the life cycle than has the artificial withdrawal of blood from a horse infected with *Dourine*. In other words, it would seem that the transference by flies in the case of sleeping sickness may have no more significance with respect to the life history of the parasite than has the direct inoculation of *Dourine* from horse to horse by means of a needle.

As we have already pointed out, the observations of Bruce, Dutton, Todd and Hanington* and others seem to indicate that the transference of sleeping sickness, when it is brought about by flies, is in the nature of a simple inoculation of blood, while it would appear that Dutton, Todd and Hanington incline further to believe that flies are not necessarily the normal means by which the propagation of sleeping sickness takes place.

They sum up the situation in this respect as follows:—"It seems "that all the results are in conformity with the hypothesis that "*Glossina palpalis* transmits *Trypanosoma gambiense*, and that it "is probably not able to do so when the space between the trans- "mitting feeds exceeds 48 hours; this conclusion is, nevertheless, to "our minds a most unsatisfactory one, if we are to regard these "Glossinae as the chief or only carriers of *Trypanosoma gambiense*. " . . . It certainly seems possible that mechanical transmission "by tsetse flies cannot alone be responsible for the rapid spread of "sleeping sickness of recent years."

These questions, however, open out a wide field of enquiry, which it is at present unprofitable further to discuss.

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* Loc. cit.

APPENDIX I

METHOD OF PREPARING AND STAINING WET FILMS, USED DURING THE FOREGOING INVESTIGATIONS

Place a very thin layer of albumen-glycerine on a clean slide. The best method is perhaps to put a drop the size of a large pin's head on the slide, and to spread this with a clean duster over the slide. On top of this layer spread a drop of blood in the usual way, and dip the slide, while wet, into the fixing solution (Flemming's strong solution was usually used). Leave it for about five to ten minutes, wash immediately in water, and pass the slide through alcohols in consecutive order, increasing by 10 per cent. at a time to absolute alcohol. Then back from absolute into 80 per cent. alcohol in which is contained iodine and potassium iodide. In order to prepare the solution of 80 per cent. alcohol containing iodine and potassium iodide, make up a concentrated solution of potassium iodide in water, and iodine in alcohol, mix them together, and add them to some 80 per cent. alcohol until the mixture becomes a dark brown colour. Leave the slide in this from five to ten minutes, and then bring it into 30 per cent. alcohol. Use for staining either aniline safranine (nach Babes), or the following solution:—Prepare a concentrated watery solution of safranine (Grübler) and a concentrated alcoholic solution of safranin, mix them in equal parts, and then add pure aniline oil. Shake from time to time, and leave the solution to ripen for three to six months. Stain in this solution for from half an hour to two hours. Wash off the safranin, and stain afterwards with polychrome methylene blue [one gramme methylene blue purissimum medicinale (Höchst) 100 c.cm. distilled water and ·5 gramme sodium carbonate, left in an incubator to ripen; the older the solution the better the staining properties]. Wash off the methylene blue, and differentiate with Unna's orange tannin, as long as the blue stain comes out. Bring the slide up through alcohols, as above, into absolute alcohol. The film has then a reddish colour. Now dip the slide into aniline oil until the reddish colour changes to a purple-blue tinge; the aniline oil takes out at the same time the excess of blue stain left by the orange tannin. Clear in xylol. Mount in Canada Balsam under a coverslip.

APPENDIX II

MODIFICATION OF HEIDENHAIN'S HAEMATOXYLIN METHOD, USED DURING THE FOREGOING INVESTIGATIONS

Fix and treat the film in the way described in Appendix I. Clear the slide from the alcohol containing iodine and potassium iodide, and pass it through successive alcohols (as in Appendix I) into water. Stain in a $3\frac{1}{2}$ per cent. solution of iron alum for one hour, wash this off, and stain with the following solution:—·5 gramme haematoxylin dissolved in 100 c.cm. distilled water, to which, after the haematoxylin has dissolved, a few drops of concentrated watery solution of lithium carbonate is added. Stain for half an hour, and then differentiate in the usual way with iron alum.

DESCRIPTION OF FIGURES

In all cases, unless otherwise stated, the figures have been drawn with a long tube Zeiss, 2 mm. apo. objective and 18 or 27 eyepiece.

PLATE XXXVIII

Trypanosoma gambiense

Figs. 1, 2, 3 from peripheral circulation stained with iron haematoxylin.

Figs. 4 and 5 stained with Breinl's stain.

Fig. 1.—Trypanosome in the resting condition. Nucleus single, not dividing. Intra-nuclear centrosome single.

Fig. 2.—Trypanosome showing early stages in division of nucleus, intra-nuclear centrosome divided; extra-nuclear centrosome also divided, new flagellum growing.

Fig. 3.—Trypanosome in the same stage as preceding. Intra-nuclear centrosome completely divided; the new flagellum is also seen during the course of its development.

Fig. 4.—Trypanosome is in the same condition as fig. 1, showing also the metabolic granules in different parts of the cell.

Fig. 5.—Trypanosome wherein two undulating membranes have been formed, showing also the metabolic granules.

Fig. 6.—Trypanosome in much the same condition as in fig. 1, but showing early stages in development of the new flagellum.

Fig. 7.—Later stage in the division, showing the mode of division of the intra-nuclear centrosome, amitotic fission of the rest of the nucleus, and the duplication of the extra-nuclear centrosome.

Fig. 8.—Trypanosome showing three flagella and three nuclei in different stages of division.

Fig. 9.—The same showing three flagella and four nuclei.

Fig. 10.—The same showing two flagella and two nuclei.

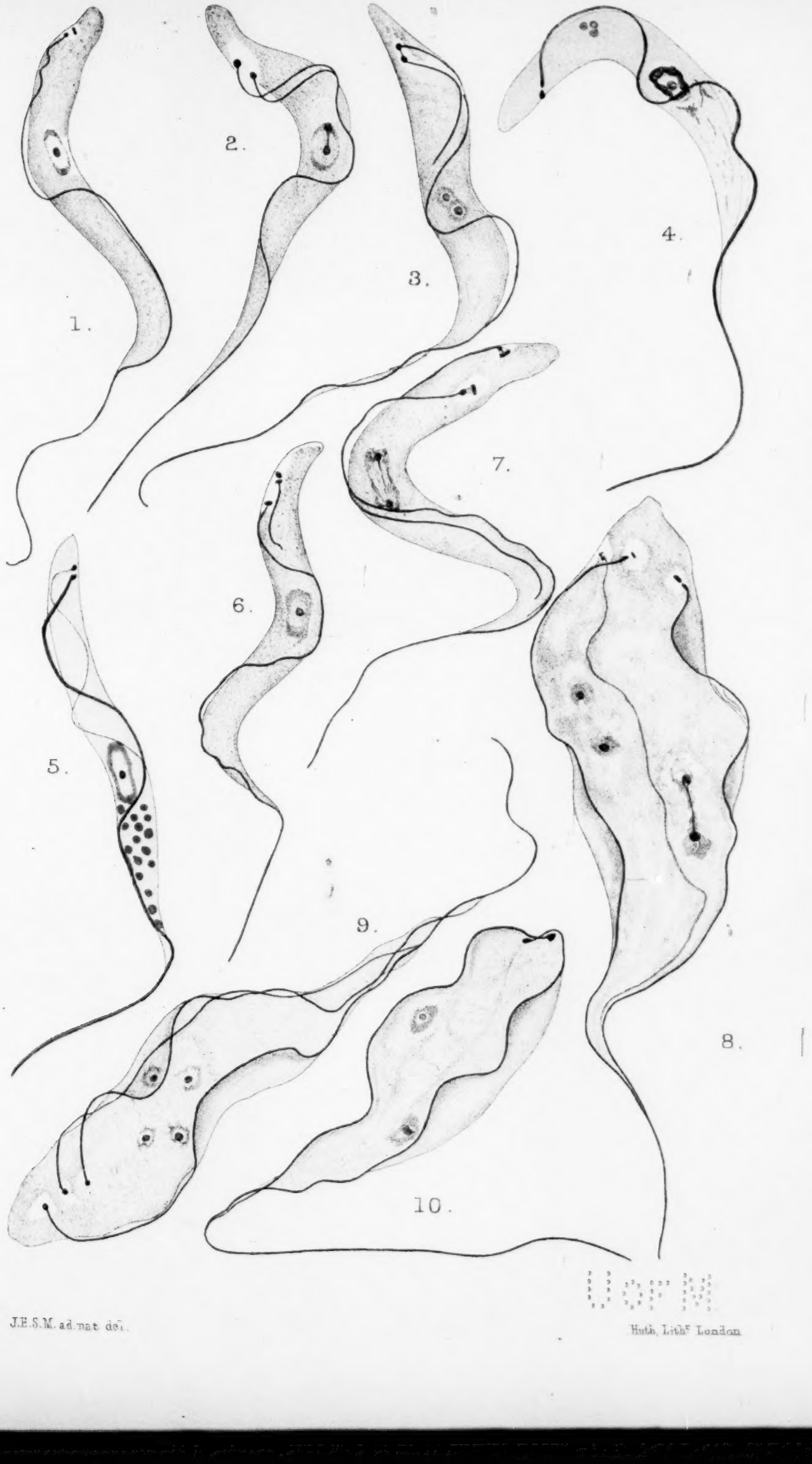

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PLATE XXXIX

Trypanosoma gambiense

Figs. 11 to 14 stained with Breinl's stain.
Figs. 15 to 21 stained with the modification of Heidenhain's haematoxylin.

Fig. 11.—Trypanosome showing division of the intra-nuclear centrosome, and the nuclear substance. Also multiplication of the intra-nuclear centrosome so as to form a group.

Fig. 12.—Trypanosome showing mode of amitotic separation of the nuclei. Multiplication of the intra-nuclear centrosome, and the formation of an independent group of these bodies. In this and the preceding figures metabolic granules are also seen.

Fig. 13.—Later stage in the fission of a trypanosome. The flagella are being detached from one another at the thin end of the cell. The cell body is dividing from this end towards the other. The nuclei are already divided. Metabolic granules are scattered throughout the spongioplasm.

Fig. 14.—Later stage in the division of a trypanosome, showing the manner in which the daughter cells separate.

Fig. 15.—Trypanosome, at one of the maximum periods of the infection, showing a single flagellum and resting nucleus, and also the origin of the black line from the extra-nuclear centrosome.

Fig. 16.—A similar stage wherein the black line has reached the neighbourhood of the nucleus.

Fig. 17.—The same.

Fig. 18.—Trypanosome showing the black line coiled upon itself towards the intra-nuclear centrosome.

Fig. 19.—Trypanosome showing early stages in the degeneration of the black line, and its later direct association with the nucleus.

Fig. 20.—Similar stage, wherein the intra-nuclear centrosome has become divided.

Fig. 21.—So-called involution stage, showing resting nucleus and multiplication of the intra-nuclear centrosome.

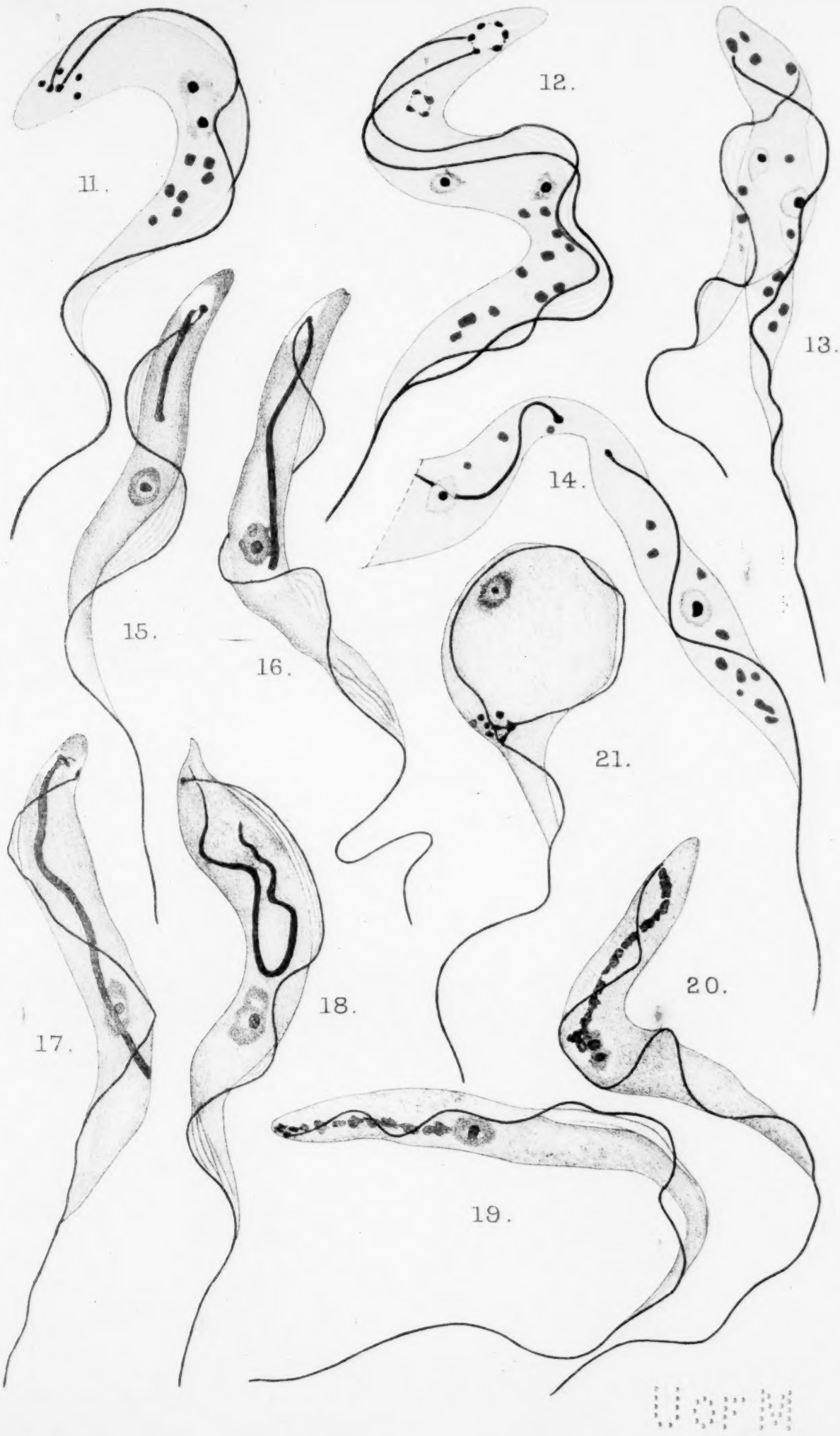

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PLATE XL

Stages in the Metamorphosis of Trypanosoma gambiense in the Organs

Figs. 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, stained with a modification of Heidenhain.

Figs. 25 and 33 stained with Breinl.

Fig. 22.—Trypanosome during the decrease of the parasites in the blood of a rat, showing alteration in the nucleus marked by the formation of a vacuole.

Fig. 23.—The same.

Fig. 24.—One of the common forms in the lung at this period, showing the same changes in the nucleus.

Fig. 26.—Low power view of trypanosome at this period, showing the detachment of the latent body from the parent cell.

Fig. 27.—Latent bodies. The nucleus is attached to a vacuole and both are surrounded by a thin film of protoplasm. The nucleus contains a single intra-nuclear centrosome.

Fig. 28.—Latent bodies. To the left the intra-nuclear centrosome is shown divided. To the right stages in the division of this body and the extrusion of one daughter element from the nucleus.

Fig. 29.—Latent bodies showing origin of a new flagellum from the intra-nuclear centrosome.

Figs. 30, 31.—Later stages in the formation of small trypanosomes from the latent bodies.

Fig. 32.—Latent body from the spleen of a rat infected with *Trypanosoma brucei*, showing nucleus, vacuole, and the origin of the flagellum from the intra-nuclear centrosome. (Compare fig. 29.)

Fig. 33.—Trypanosome showing early stages in division of the nucleus.

Fig. 34.—Trypanosome drawn to show the Schaumplasma structure of the protoplasm.

Figs. 34 to 36.—The same.

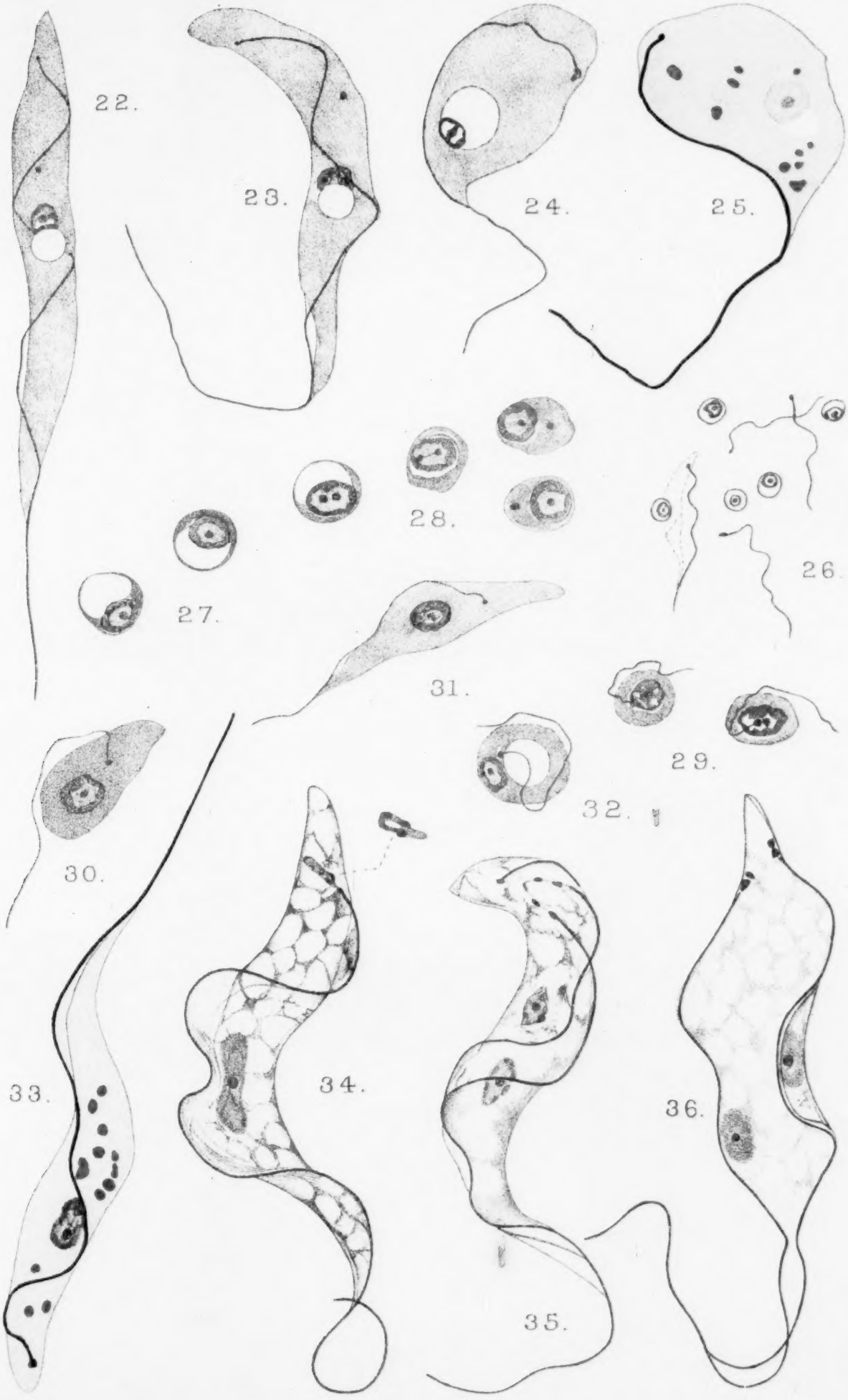

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PLATE XL

Stages in the Metamorphosis of Trypanosoma gambiense in the Organs

Figs. 22, 23, 24, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, stained with a modification of Heidenhain.

Figs. 25 and 33 stained with Breinl.

Fig. 22.—Trypanosome during the decrease of the parasites in the blood of a rat, showing alteration in the nucleus marked by the formation of a vacuole.

Fig. 23.—The same.

Fig. 24.—One of the common forms in the lung at this period, showing the same changes in the nucleus.

Fig. 26.—Low power view of trypanosome at this period, showing the detachment of the latent body from the parent cell.

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Fig. 34.—Trypanosome drawn to show the Schaumplasma structure of the protoplasm.

Figs. 34 to 36.—The same.

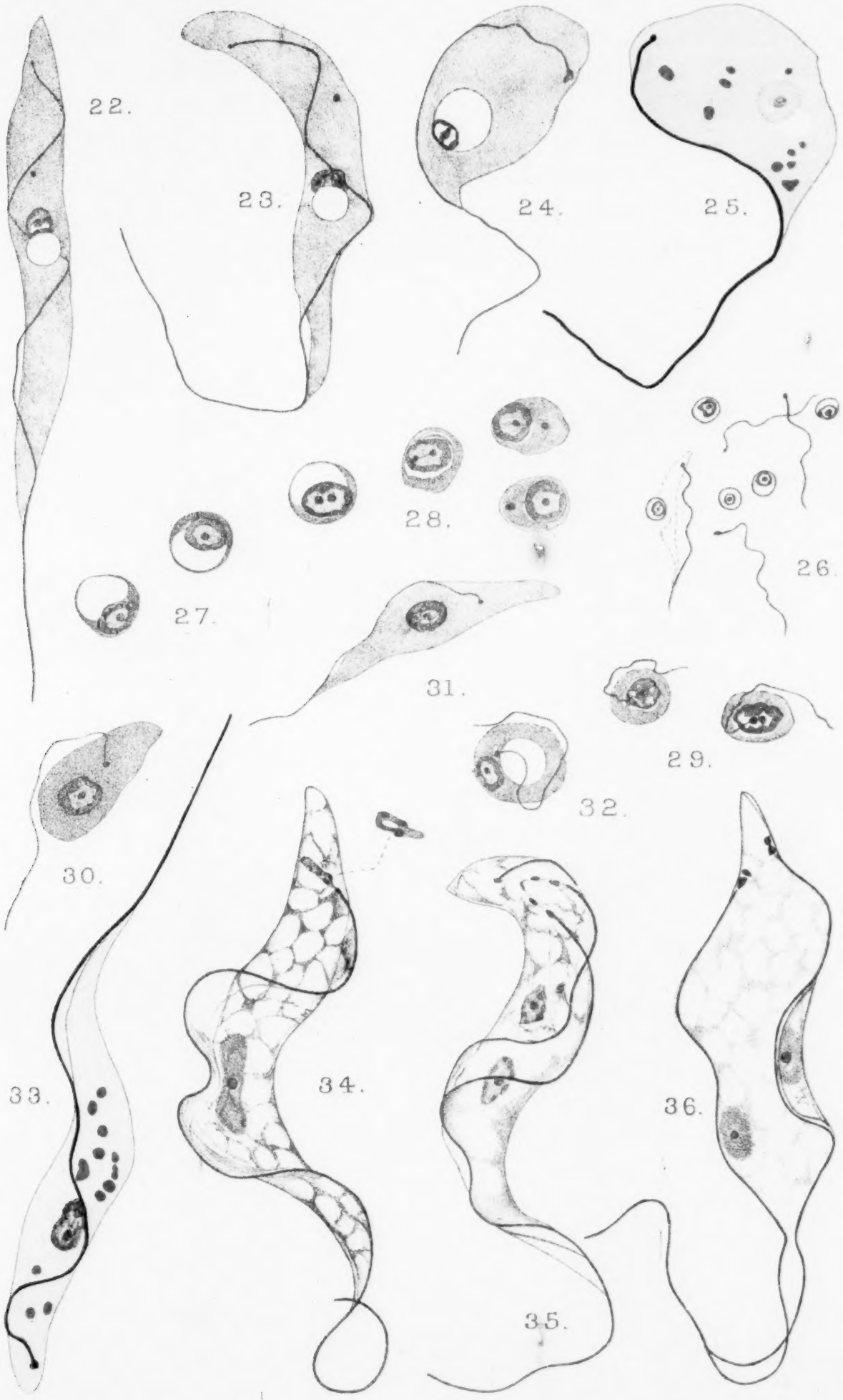

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PLATE XLI

Trypanosoma gambiense and Trypanosoma brucei

Figs. 37-41, *T. gambiense.*
Figs. 42-46, *T. brucei.*
Figs. 37, 38, 40, 41, 42, 43, 45, 46, Breinl's stain.
Fig. 44, Heidenhain.

Fig. 37.—Trypanosome in the blood of a rat after treatment with atoxyl, showing rounding up of the cell body.

Fig. 38.—Further stage in this process, flagellum is still attached. A slight modification of a membrane is apparent round the periphery of the cell.

Fig. 39.—The same, flagellum not visible.

Fig. 40.—Later stage in the formation of the cyst. The membrane more apparent.

Fig. 41.—Trypanosomes killed by atoxyl in the blood.

Fig. 42.—*Trypanosoma brucei* in resting condition, showing structure of the nucleus and relation of the schaumplasm.

Fig. 43.—The same, showing the division of the extra-nuclear centrosome.

Figs. 44 to 46.—Later stages of division.

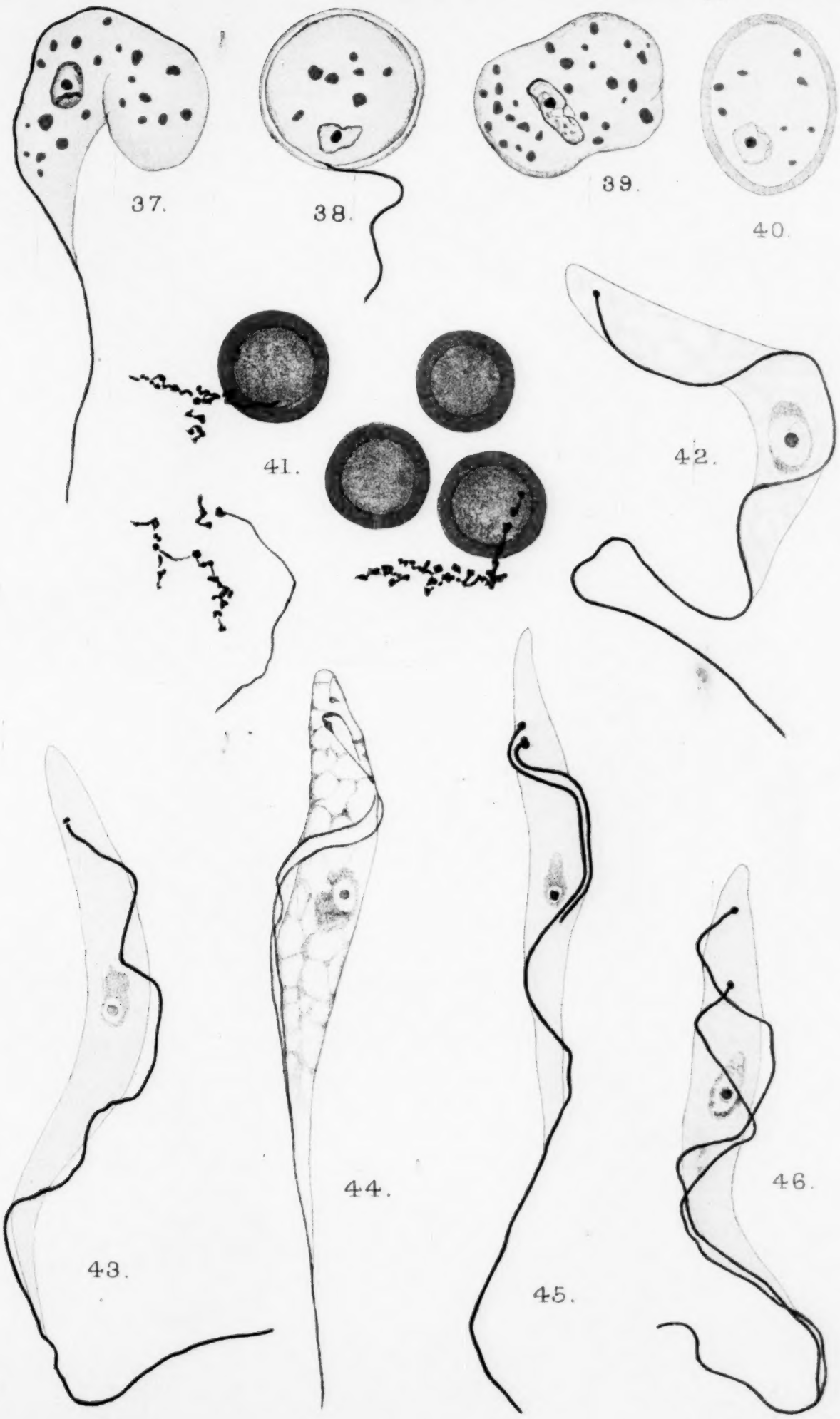

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PLATE XLII

Trybanosoma equinum

Figs. 47, 49, 50, 51, 52, 53, 54, stained with Breinl.
Fig. 48 stained with modification of Heidenhain.

Fig. 47.—Resting stage of the trypanosome.

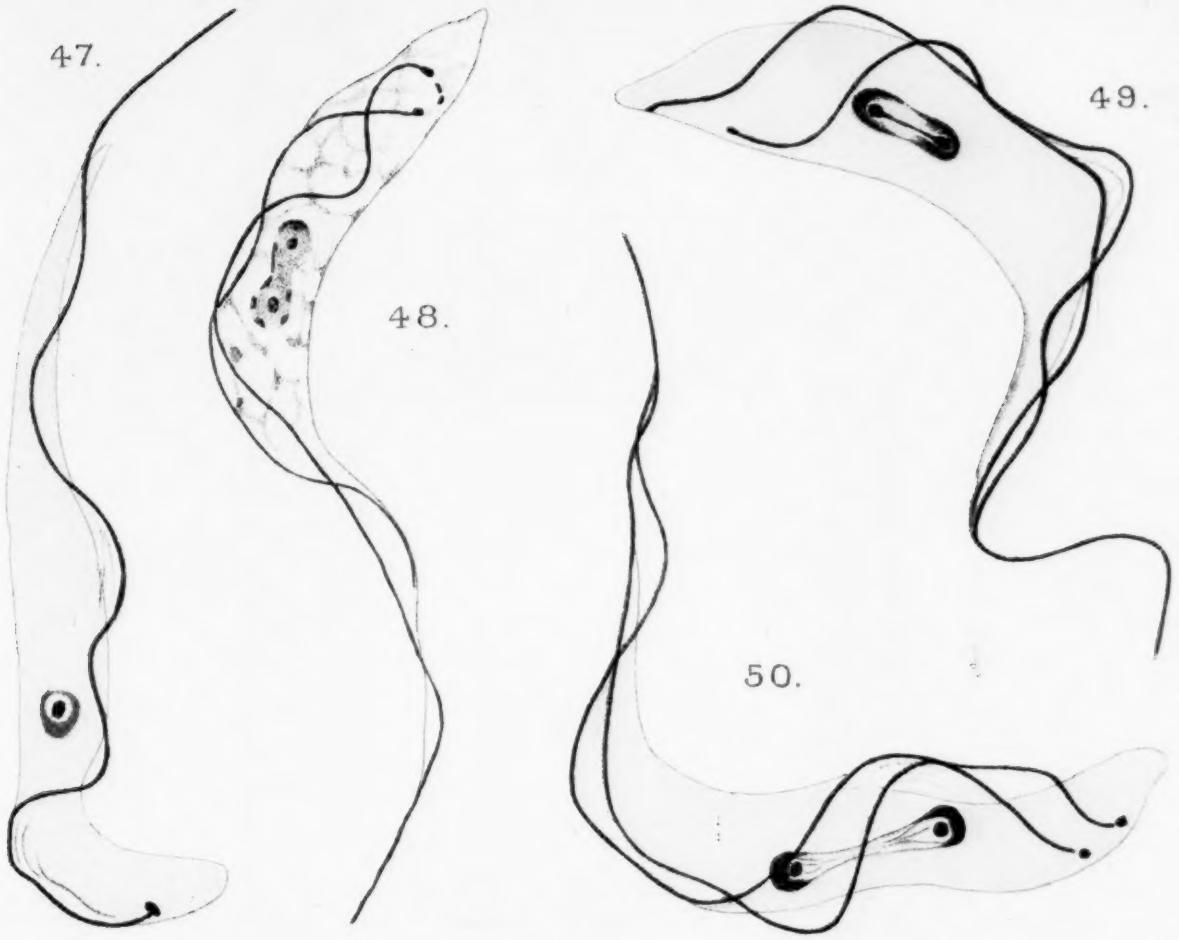
Fig. 48.—Stage showing formation of the new flagellum and division of the nucleus.

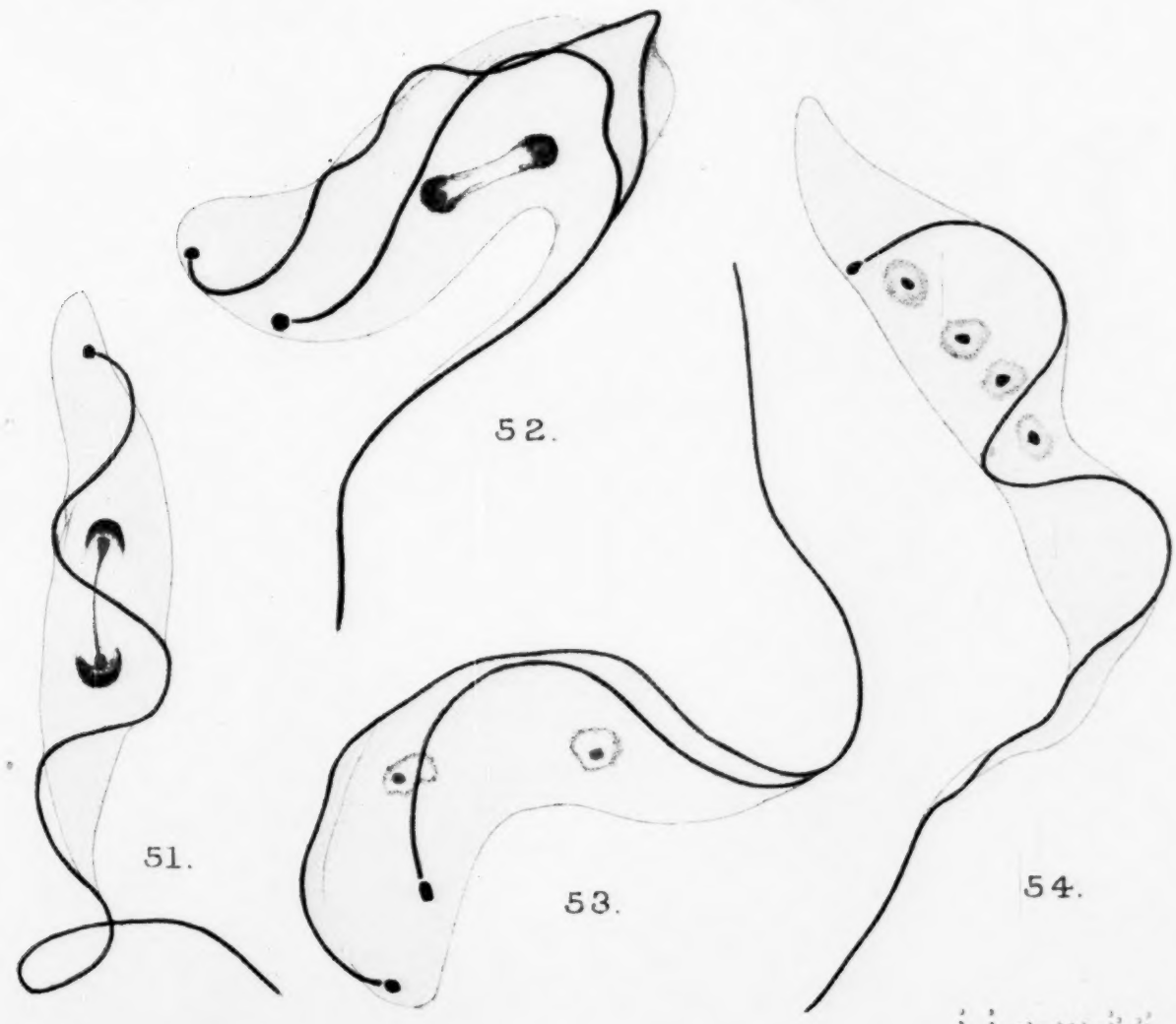
Fig. 49.—Trypanosome showing details of the division of the intra-nuclear centrosome and the nuclear substance.

Figs. 50, 51.—Trypanosomes showing later stages of the same process.

Figs. 52, 53.—Trypanosomes showing still later stages in the division of the nucleus and the characters of the intra-nuclear centrosomes.

Fig. 54.—Trypanosome wherein the nucleus has divided into four constituents, although there is only one flagellum.





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